FREEZE-THAW EFFECT ON SOIL MICROBIAL ACTIVITY WITH BIOCHAR APPLICATION IN SUBARCTIC SOIL

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A

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Abstract

Alaska has limited agricultural production due to extreme climatic conditions and weakly developed soils, which affect productivity. In higher latitudes, freeze-thaw cycles are common and influence soil biology and nutrient dynamics, offering a unique opportunity to investigate the use of soil amendments like biochar to enhance native biota and soil's intrinsic properties. Biochar for this study was produced from locally harvested black spruce (*Picea mariana*), using a fixed bed pyrolysis unit. The production of biochar was electronically controlled with temperatures kept at 550°C, and residence times manipulated by a mechanical auger, in order to yield five distinct biochar products. Chemical analyses showed differences among the biochar samples, including cation exchange capacity (CEC), micronutrients and pH. To evaluate the influence of each biochar on higher latitude native soils and biota, a response surface model was employed to design a set of experiments that measured CO_2 accumulation during a 15-day freeze -thaw cycle. Microbial activity during this experimental phase was monitored before and after freeze-thaw. Results of this study demonstrated that cultivated soils amended with biochar showed higher microbial activity before and after freeze-thaw. Forest soil on the contrary showed no significant results when amended with biochar. These results on different microbial activity were likely due to the amounts of organic carbon present in each soil type. The study serves as an evaluative tool for determining the impact that biochar may have in subarctic regions of the US that have limited agricultural potential as a result of climatic and native soil conditions.

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Chapter 1 Introduction

1.1 Alaska's Agricultural Facts

Alaska is a large geographical region characterized by an extended winter season from September through April, when most soils could be covered in snow or water could be frozen in some areas. The result is a short growing season, that averages 105 days for the South Central and Interior regions of the state, where most agricultural production occurs (http://www.uaf.edu/ces/pubs/catalog/ Accessed 9/20/2012). The short growing season is met with vigorous biomass growth as a result of long daylight hours, which often results in adequate production of crops, including potatoes, beets, barley and hay ("State Fact Sheet: Alaska, n.d" Retrieved January 28, 2013. http://dnr.alaska.gov/mlw/factsht/). Nonetheless, it is estimated that 90% of the food needs for the 698,473 residents of the state is imported (U.S Census Borough April 2010. *State & County quick facts: Alaska.* Retrieved January 28, 2013. http://quickfacts.census.gov). Also, due to the limitations in production outside of the frost free season, controlled environments, (e.g. greenhouses) have been successfully producing tomatoes and specialty crops ("Fact Sheet: Alaska, n.d" Retrieved January 28, 2013. http://dnr.alaska.gov/mlw/factsht/), but with limited market penetration. The geographical location of the State adds considerable cost to the transportation of food, as well as agronomic inputs, such as fertilizers, herbicides, fungicides and other products necessary for agriculture in the state. Food security issues are being addressed by Alaskans with an increase in the number of incentive programs to farmers, and a growing interest among individuals to invest in small farms and gardens statewide ("Agriculture Information, n.d". Retrieved January 28, 2013.

http://dnr.alaska.gov/mlw/factsht/). The demand for locally grown produce is gradually increasing, and along with it, a need for more sustainable practices in the agricultural

sector. From an agricultural standpoint, Alaska not only has some unique climatic conditions that affect agriculture, but its weakly developed soils present a challenge to producers. Soils in high latitudes are naturally low in fertility, structure and poor in soil fauna (Buol et al., 2011); which makes producers rely on fertilizers and soil amendments. The amendments most commonly used for gardening are vermiculite, potting soil, peat moss and others which are usually imported and have become subject to upwards economic pressures due to transportation costs (http://www.commerce.state.ak.us/dca/pub/Fuel_Report_2012_July.pdf. Retrieved 4/20/2013). As the population grows, increasing food demand will require augmenting the use of nutrients and soil amendments to ensure higher yields. While there is willingness to reduce the use of synthetic fertilizers, generally natural alternatives continue to be lacking at a local level. Alaska's growers are interested in implementing sound practices for their farming operations, especially those that can be created from local resources.

1.2 Biochar Properties

Biochar is a soil amendment that has the potential to address soil quality issues like pH, poor structure, nutrient leaching, and other concerns in soils with poor physical and chemical characteristics that decrease plant growth and production (Lehmann, 2007).

Biochar is biomass that has undergone thermal decomposition through a process deficient of oxygen called pyrolysis (Lehmann and Joseph, 2009). The biochar formation process involves the release of the volatile species from the biomass particle, leaving behind a carbonized, highly porous skeleton that has unique physical and chemical properties that enhance soil structure and biota (Atkinson et al., 2010). Biochar is mainly comprised of aromatic compounds, which are resistant to abiotic and biotic degradation; consequently it is a highly stable material and becomes a recalcitrant component in the soil (Lehmann and Joseph, 2009). These attributes, coupled with the porous nature of the biochar particles result in an increase in microbial activity due to the availability of labile C specially found in young biochars (Smith et al., 2010). In addition increase of water, nutrient retention and pH alteration to more neutral levels on soils has been positively correlated after biochar application (Lehmann and Joseph, 2009).

1.3 Biochar Production

Biochar properties are affected for the most part during its formation in pyrolysis and the main driver for this is heating rate (Lehmann, 2007). Using the heating rate as a basis, one can categorize two types of pyrolysis: slow and fast. In slow pyrolysis the heating rate, as determined by the combination of temperature and retention time results in the biomass particles reaching the target temperature (300C° to 400C°) in several minutes. By contrast, fast pyrolysis systems heat the particles up to the target temperature (500C° to 600C°) in several seconds, with the resulting products being bio-oil and biochar respectively (Brown et al., 2011). At higher heating rates, some biochar exhibit higher

CEC, porosity (Lehmann, 2007) and more aromaticity which in turn increases its stability in the soil (Kloss et al., 2012); this provides more benefits to above ground biomass. Biochar can be created with any source of organic feedstock which can include animal manures (Hass et al., 2012), cornstover, nut shells and wood (Lee et al., 2010; Özçimen and Ersoy-Meriçboyu, 2010). All feedstocks that have been evaluated in the literature show some level of benefit, regardless of the source of the organic material. However, some of the perceived benefits may have a strong correlation to the soils to which the biochar was applied to.

1.4 Biochar Research

Biochar has been extensively researched in the tropics, where soils are highly weathered and have poor fertility. The application of biochar in these soils have shown a direct effect on pH, nutrient retention and yield (Major et al., 2010). Other studies have been undertaken in temperate regions with satisfactory results among biochar and soil properties and biota (Kolb et al., 2009; Smith et al., 2010). Healthy soils on the other hand, are fertile and generally have the following characteristics: high organic matter content which has intense effects on soil functions like to retain water and nutrients, support cation exchange capacity and others. Adequate pH ranges between 6.5 and 7, at which the soil is neither acidic nor alkaline, nutrients are available to plants, toxins are unavailable and beneficial microorganisms can thrive. Good soil structure allows water to drain, oxygen to penetrate and plants roots to move freely in the soil, which creates a well-functioning biological soil ecosystem. All these qualities are present in a fertile soil with good development. Therefore addition of biochar may not be necessary nor would it be advantageous for soils that already have well-established soil chemical, physical and biological properties.

Although the rate of decomposition could be slow in high latitudes and soil organic carbon can easily accumulate, soil organic carbon can also decrease substantially when forest land is cleared for agriculture (Lal, 2005; Sparrow et al., 1992). When forest soils are converted into agricultural soil organic carbon can be reduced by 20 to 50% and have direct effects on soil properties like C:N ratio, soil moisture, organic matter decomposition and can disrupt the overall nutrient cycle (Lal, 2005). Agricultural soils can benefit from the addition of biochar when the soil carbon stocks have been depleted or reduced.

1.5 Study Scope

Little is known about biochar's effectiveness in high latitude agricultural settings, therefore, evaluation is necessary in a controlled framework, to provide farmers accurate and reliable data about this technology and best management practices. The goal of this project is to determine the viability of using biochar in high latitude agriculture applications, by creating and testing black spruce biochar from local Alaskan resources, and evaluate its impact on the soil microbial community, in particular as it undergoes repeated freeze-thaw cycles. The freeze thaw effects and the sub-arctic soil applications have not been described in the literature before, nor has the production of biochar using Alaskan fire-killed black spruce biomass.

The current document is organized by chapters written in the style of peer-reviewed publications prepared as a result of the current study. Chapter 2 describes the soil microbial effects of adding different biochar ratios to two distinct Alaskan subarctic soils that are subject to freeze-thaw cycles.

Chapter 3 is the unifying conclusion of the thesis, summarizing the results found in the preceding chapters, including suggestions for further research.

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Chapter 2 Freeze-thaw effect on soil microbial activity with Biochar application in subarctic soils

2.1 Introduction

High latitude includes regions where traditional agricultural practices have limited, and are under dynamic stress due to climate change and a projected increase in population. Some climate change scenarios (Olesen, 2008), would benefit traditional cropping systems in circumpolar regions. However to a great degree, the region would continue to be a net importer of food due to the weak development of the soil, as is the case for most of Alaska. Challenges to meet the potential growth of larger scaled agriculture are many, but soil quality and distribution are perhaps the main factors to consider. Soils in higher latitudes are relatively young and weakly developed (Buol et al., 2011). Soil forming factors and processes occur with a different intensity and recurrence in these regions, and the effects of climate are major factors in the development of soils; temperature, precipitation, radiant energy and aspect of the land affect the physical and chemical processes of soil development in circumpolar regions (Ping et al., 2005). Temperature has additional effects on biological activity, decomposition rates, evapo-transpiration and frost free days required for agriculture to be effective in the region.

In some northern latitude regions, severe low temperatures for long periods of time support the formation of perennial ice under thick accumulations of organic matter, leading to permafrost formation technically known as Gelisols soils (Soil Survey Staff, 1999). These soils are difficult to farm because of the prevalence of a shallow, cold, restrictive layer of perennial ice that influences the soil temperature conditions and impedes water drainage in most cases. Other soil types found in the region are also affected by low evapo-transpiration and slow decomposition rates of organic matter due to the low temperatures which result in the prevalence of a wet highly organic soil or Histosols (Soil Survey Staff, 1999). These soils cover large areas in subarctic and arctic regions and are not suitable for agriculture due to their high wet organic matter content but play a valuable role in wetland ecosystems. An exception can be found in south facing aspects that receive higher solar radiation and are well drained in higher latitudes, where Inceptisols or Entisols (Ping et al., 2005) occur, which are the most promising soil types for agriculture. Although these soils are weakly developed, the existence of biota contributes to nutrient cycling, profile mixing by bioturbation, increased fertility and promoting soil development to an extent (Buol et al., 2011) Yet, the formation processes are slow in these soils due to low temperatures, low precipitation in some areas and resistant parent material. In Alaska, major agricultural settings take place under Inceptisols and some soil series under this soil order are recognized as soils of local importance for agriculture. However this soils are not naturally fertile therefore amendments are frequently necessary (http://www.ak.nrcs.usda.gov/soils/soilslocal.html last accessed 4/11/13.)

Little research has been conducted on the mechanisms for enhancing agricultural productivity in higher latitudes. The need to apply new innovative practices and use sustainable products to protect agricultural soils in higher latitudes is therefore crucial if the region is to support food production for its current and projected future population. A promising agricultural amendment that can be manufactured locally without fossil based resources, and that has demonstrated positive effects at the physical, chemical and biological levels in agricultural soils found in temperate and tropical regions of the world is biochar. Biochar is biomass that has undergone thermal decomposition in an oxygen deficient process (Lehmann and Joseph, 2009). The biomass transformation occurs when the volatile species contained in the biomass, are released from the solid particles as heating occurs, leaving behind a carbonized, highly porous skeleton. The carbon rich biochar is mainly comprised of aromatic compounds and other functional groups that makes it resistant to biotic and abiotic decomposition and provides long-term soil stability (Cheng et al., 2006). This porous structure has significant effects on the habitat of soil microorganisms, promoting bacterial and fungal colonization (Steiner et al., 2008; Warnock et al., 2007). Biochar also has been shown to exhibit positive effects in the retention of water, nutrients and air on its micro and macro pores at root level (Lehmann and Joseph, 2009). These qualities of biochar make it an efficient soil enhancer for depleted or poor soils, and a medium for greenhouse plants by improving soil properties and its ability to support agricultural practices in different world regions (Graber et al., 2010; Husk and Major, 2011; Major et al., 2010).

The major influences of biochar on soil chemical properties include, but are not limited to, pH buffering, increase cation exchange capacity, some nutrient addition and increase in soil biological activity (Lehmann and Joseph, 2009). These enhanced properties have been well documented in tropical soils and to a lesser extent in temperate soils, but have not yet been evaluated in subarctic soils. The most compelling evidence for biochar's effect on soils comes from studies conducted on temperate region soils were biochar additions indicated a significant effect on microbial biomass, activity and nutrient availability (Kolb et al., 2009). In contrast, for the subarctic region, most information available is limited to evaluations of post fire charcoal on the ecological effects of boreal forest soils (Olle et al., 1996). This type of carbon plays a significant role in the boreal forest region ecosystem, and has been demonstrated to have a significant effect on the adsorption of secondary metabolites. Ecologically, these metabolites, mainly comprised of phenolic compounds produced by ericaceous vegetation, can negatively affect forest rejuvenation (Wardle et al., 1998) The wildfire charcoal's large sorption capacity has the ability to adsorb large amounts of these phenolics for many years, allowing nutrients and microbiological activity to foment plant growth (Olle et al., 1996), and affecting boreal forest ecosystems and boreal forest converted land. However, the application of engineered biochar has not been evaluated in the region's soils.

Soils in high latitudes are seasonally affected by freeze-thaw cycles, which have been shown to influence soil microorganisms, nutrient cycling, and soil structure (DeLuca et al., 1992; Schimel and Clein, 1996). Physically, during freeze –thaw, soil aggregate stability decreases especially when the soil moisture is high (Six et al., 2004), and destruction of macro and micro aggregates occur during this repeated freeze-thaw cycles

(Unger, 1991). At the nutrient level, freeze-thaw cycles produce and increase in N mineralization and mineral N flush (DeLuca et al., 1992). There is also a C flush during freeze-thaw from decomposed microbial biomass that accounts for up to 65 percent of the total C measured after a cycle is complete (Herrmann and Witter, 2002). In subarctic soils, microbial activity is significantly affected by freeze-thaw cycles, which occur in late fall and early spring. Microbes surviving in soils under frozen conditions are thought to survive on the surface layers of soil particles where significant amounts of unfrozen water can persist as films, even at very low temperatures (Kurganova et al., 2007; Walker et al., 2006). Furthermore soil microbes suffer a detrimental loss in population during freeze-thaw. The cells of microorganisms are destroyed during the repeated freeze-thaw cycles which further result in an increase of soluble sugars and amino acids in the soil (Ivarson and Sowden, 1966; Morley et al., 1983). This soluble organic material becomes readily available to the surviving microbes during thaw, contributing to a CO_2 burst produced by microbial activity as measured in respiration experiments (Skogland et al., 1988; Walker et al., 2006).

The influence of different types of biochar and their effects on soil environments is not fully understood. Region specific research is needed to understand the positive and negative interactions among biochar and soil microorganisms for agricultural purposes. Given that biochar additions to soils provide additional surface area that are good microbe habitats, additions to cold-region soils could have the potential effect of increasing both the amounts of unfrozen water and cold-surviving microbes, resulting in changes to the microbial and nutrient cycling capacity of cold-soils. In this study we examined the effects of adding different biochar ratios and types on microbial activity respiration of subarctic cultivated and forest soils of south-central Alaska influenced by freeze-thaw cycles.

2.2 Materials and Methods

Cultivated and forest soils of sub-central Alaska were collected and amended with different biochar rates. Five different types of biochar were created at different residence times for this study. Samples were incubated and connected to a respirometer to measure microbial activity before and after freeze-thaw.

2.2.1 Model

A response surface model was developed specifically for this study to evaluate the effect of biochar on microbial activity on subarctic undisturbed forest and cultivated soils. The experimental design framework was based on a response surface model constructed using Design Expert v7 software. The response surface model was built using a biochar to soil ratio and the retention time of the original biomass inside the pyrolysis reactor ($550^{o}C$) used in the production of the biochar as the independent variables. For the design, the actual experimental parameters ranged from 160.7 sec to 73.6 sec residence time that corresponds to the max and min revolution per minute (RPM) of the pyrolysis unit that was electronically controlled. The biochar: soil ratio ranged from 2 to 20 percent or the equivalent of 0.95 g to 11.25 g of biochar to 50 g of soil. The design parameters are presented on Table 1. This resulted in actual experiments using five distinct retention times and biochar to soil ratios. To evaluate the effectiveness of biochar and the effect of freeze-thaw, the response used for the model was the CO_2 accumulation from microbial respiration measured before and during freeze-thaw for each of the experiments under the conditions summarized in Table 1.This experimental set up was the same for both soils (cultivated and forested) and both were evaluated using the same procedures.

Table 1. Response surface model experimental set up conditions met for agricultural and forestry soils.

Run	Pyrolysis units RPM	Retention time (seconds)	Biochar (g)	Biochar to soil % based on 50 g of soil
1	16.2	97.3	6.25	12.5
2	16.2	97.3	0.95	1.9
3	10.8	134.4	2.5	2.5
4	21.6	81.97	2.5	2.5
5	16.2	97.3	6.25	12.6
6	23.8	73.6	6.25	12.5
7	21.6	81.97	10	20
8	16.2	97.3	6.25	12.5
9	8.56	160.7	6.25	12.5
10	10.8	134.4	10	20
11	16.2	97.3	6.25	12.5
12	16.2	97.3	11.25	22.5
13	16.2	97.3	6.25	12.5

2.2.2 Biomass feedstock characterization

Black spruce (*Picea mariana)*, was locally sourced at the University of Alaska Fairbanks Matanuska Experiment Farm in Palmer, Alaska. The biomass was ground to 2 mm particle size using a Thomas Scientific Wiley Mill, Model 5, and dried for 48 hours at constant temperature of 60 C° in a forced air-drying oven. Original black spruce samples were evaluated for composition prior to conversion into biochar. Analyses included moisture content ASTM D-444-92, extractives ASTM 1108-96, Klason lignin ASTM D1106-96, and carbohydrate content was calculated by difference. Elemental C, H, N and O percent composition was determined using a LECO Elemental Analyzer Model Truspec following instrumental determination on CHN ASTM D5373-93. Functional chemical groups were evaluated by FTIR (Fourier Transform Infrared) spectroscopy using a Thermo Nicolet IS 10 instrument with an ATR probe.

2.2.3 Biochar production

Biochar was produced in a pilot scale fixed bed pyrolysis unit, with a capacity of 1 kg per hour. The independent variables affecting biochar properties, namely temperature and retention time were electronically controlled in the unit. Temperature was maintained at 550^{o}C and the retention time varied between 73 and 160 seconds according to the response surface model. The retention time corresponds to the time the particles spent at the heated zone (550^{o}C), where biochar formation occurred. To produce biochar, the wood flour was introduced into a feeder hopper, sealed and flushed with argon gas to evacuate any oxygen from the reactor chamber. A mechanical agitator was used to move particles into the main reactor chamber, where an electronically controlled auger was used to move the flour through the heated region at the desired speed to match the retention time of the experimental design. The condensable and non-condensable gases were separated from the solid biochar though a condenser unit and the solid materials were collected by means of a sealed, independently valved, gravity fed chamber. The biochar was maintained in sealed glass jars at room temperature until their use in the characterization and soil microbial experiments.

2.2.4 Biochar characterization

Biochar was characterized for elemental composition using a LECO CHN Truspec analyzer to establish the C, H, N and O baseline conditions using ASTM D5373-93. Functional chemical groups were analyzed for the produced biochar using a Thermo Nicolet IS 10 FTIR instrument, in ATR mode, and spectra were compared with published reports and the original raw wood to elucidate composition and changes between the samples. Available nutrient in biochar were determine by Mehlich-3 extract quantified by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Perkin Elmer Optima 300 XL instrument. Electrical conductivity was measured by the paste method and pH by the water 1:1 method. Cation exchange capacity was determined by 1N NH_4OAc (ammonium acetate) saturation and steam distillation; exchangeable bases extracted by 1N NH4 OAc were measured by ICP-AES. Surface area of the five biochar types was conducted first by determining the apparent density of each sample according to Standard Test Method for Apparent Density of Activated Carbon D 2854. Three replicates per sample were taken and the average was calculated to determine the amount of material to be use in the butane analysis. The butane analyses of the biochar samples were determined according to the Standard Test Method for Determination of Butane Activity of Activated Carbon D 5742. Two replicates for samples were conducted for this test. Scanning electron microscope images were of each biochar type. Samples were sputter (gold) coated on a Ladd coating unit before being analyzed with a ISI-SR-50 Scanning Electron Microscope isautomated with iXRF software/hardware and an e2v SSD EDS detector.

2.2.5 Soil sampling and analysis methods

Actively cropped and undisturbed forest soils were collected from the Matanuska Experiment Farm in the summer of 2011. Soil samples from the surface 10 cm were randomly collected from selected areas of both cropped and forested soils. Soils of both sites are classified as Inceptisols of the Knik soil series (silt loam coarse-silty over sandy or sandy – skeletal, mixed superactive Typic Haplocryepts). The cultivated site was under Timothy grass for 15 years with a regular early spring fertilization of 89 Kg/ha P_2O_5, 106 kg/ha $(NH_4)2SO_4$, 77 kg/ha K_2O and 136 kg/ha of urea and no tillage practices were applied during this period. The forest site was a mix of paper birch (*Betula neoalaska*), white spruce (*Picea glauca*), and aspen (*Populus tremuloides*). Forest and cultivated soil samples were sieved through 2 mm mesh and refrigerated until mixed with biochar.

Soils pH (water 1:1) was measured using an Orion pH SensorLink electrode. Electrical conductivity was determined in a saturated paste using an YSI scientific Model 35 conductance meter. Soil available P, and micronutrients were measured in Mehlich-3 extract by inductively coupled atomic emission spectroscopy (ICP-AES). Available N (NH_4^+-N plus NO_3^--N) was measured in a 2N KCl extract by colorimetry using an Orion Scientific continuous flow auto analyzer. Exchangeable bases and CEC (extracted by N

NH4 Ac then measured by ICP-AES). CHN percent ratio was performed using LECO CHN Truspec analyzer. All soil analysis was done following the UAF Plant and Soil Analysis Laboratory methods manual (Michaelson et al., 1995).

2.2.6 Microbial activity

Table 1 summarizes the experimental setup conducted for cultivated soils and forest soils. Each experiment was based on a baseline 50 g soil assay to which the respective biochar volume was added, based on the response surface model. The samples were brought to field capacity to establish appropriate level of moisture after being thoroughly mixed. Glass jars were sealed and connected to a Columbus Instruments Microoxymax 8210-CO_2 respirometer gas analyzer. CO_2 accumulation was measured as micrograms per kg of soil per hour over 6 days at room temperature (12-17 ^oC). After 6 days, the samples were incubated in a glycol bath for 7 days at 0 ^oC and for 8 days in a freezer at -17 ^oC to simulate freeze-thaw conditions in sub-arctic environments, then they were taken out of the freezer and connected back to the respirometer, head space was purged before measuring respiration to measure the microbial activity as CO_2 emitted. The respiration was measured during the thaw cycle and for six more days at room temperature. The approach for this procedure is similar to other research that studied microbial response to freeze-thaw (Schimel and Clein, 1996; Skogland et al., 1988) but with modifications as noted, for this particular experiment.

2.3. Results

2.3.1 Biomass feedstock characterization

The chemical analysis of black spruce biomass on a dry basis at the time of conversion results was: 48% C, 0.12% N, 6.25% H, 5.2% extractives, 33% lignin, and 58.6% carbohydrates.

2.3.2 Biochar characterization

2.3.2.1 Chemical composition and nutrient content

Table 3 summarizes chemical composition data of the five biochar created based on the experimental design set up. The five biochars have some slight differences in their chemical composition but not major distinctions. It is important to note the pH for all five biochars fall in the acidic range. Carbon and N are higher for all biochars than the original biomass sample but H is lower. The CEC is lower on biochars with the longest residence time 160.7 and 134.4 sec specifically; EC is higher on biochars with shortest residence time.

Table 2. Chemical composition of the five biochar types used in this experiment

Biochar residence time (sec)	**pH**	**EC dS/m**	**CEC cmol/kg**	**Carbon %**	**Nitrogen %**	**Hydrogen %**
(1) 160.7	4.73	0.12	12.15	68.66	0.28	3.58
(2) 134.4	5.06	0.11	9.77	70.78	.25	3.68
(3) 97.3	4.55	0.15	17.36	67.84	.19	3.89
(4) 81.97	4.29	0.2	16.49	64.96	.32	4.35
(5) 73.65	4.77	0.17	17.07	67.07	.23	4.55

Mehlich 3 extractable nutrients are given in Table 4 for the five biochars in the order shown on Table 3. Values are consistent for all biochars with exception of biochar five that has higher amounts of P, K, Ca, Mg, and Zn. Biochar three and four show some unusually large amounts of Fe.

Table 3. Mehlich 3 extractable nutrients of black spruce biochars

Biochar	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})
1	<1	44	100	4	198.6	166.6	5.8	214
2	<1	40	62	<1	128.6	113.0	2.6	178
3	<1	38	64	4	168.0	160.8	10.8	1018
4	<1	48	78	6	176.0	161.6	7.0	656
5	130	172	111	13	534.5	558.8	6.1	349

2.2.3.2 FTIR analysis for biochar and black spruce

Black spruce biochar types created for this experiment and original sample were analyzed by FTIR spectroscopy and summarized in Table 5. The recorded transmission mode is between 4000 and 800 cm $^{-1}$ for all biochars, and the collected spectra are presented in Figure 1.

Table 4. Functional groups of biochar and original biomass identified by FTIR analysis

Wave numbers (cm^{-1})	Functional groups
3300 and 3800 cm^{-1}	0-H stretching
1500 and 1700 cm^{-1}	aromatic C=C stretching
800 and 860 cm^{-1}	aromatic C-H stretching
1109 cm^{-1}	aliphatic ether C-O stretch
1200 cm^{-1}	CO stretching
2900 cm^{-1}	aliphatic C-H stretch
1026 cm^{-1}	Aliphatic ether C-O and alcohol C-O stretching
3346 cm^{-1}	O-H stretch
1265 cm^{1}	C-C and C-O stretch in guaiacyl

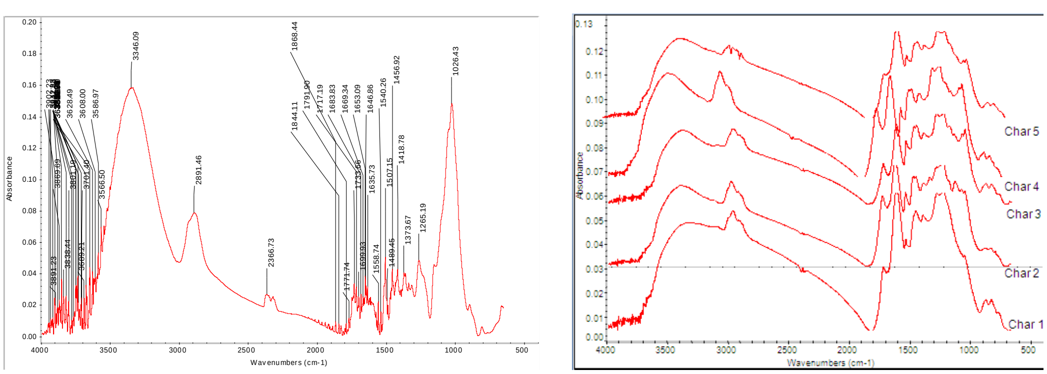

Figure 1. FTIR spectra of the black spruce sample and biochar one, two, three, four and five

2.2.3.3 Surface area and SEM images

The percentage of butane activity and SEM images for each respective biochar type is shown on Figures 2 to 7. Butane adsorption was higher for biochars one, two and five but lower for biochars two and three.

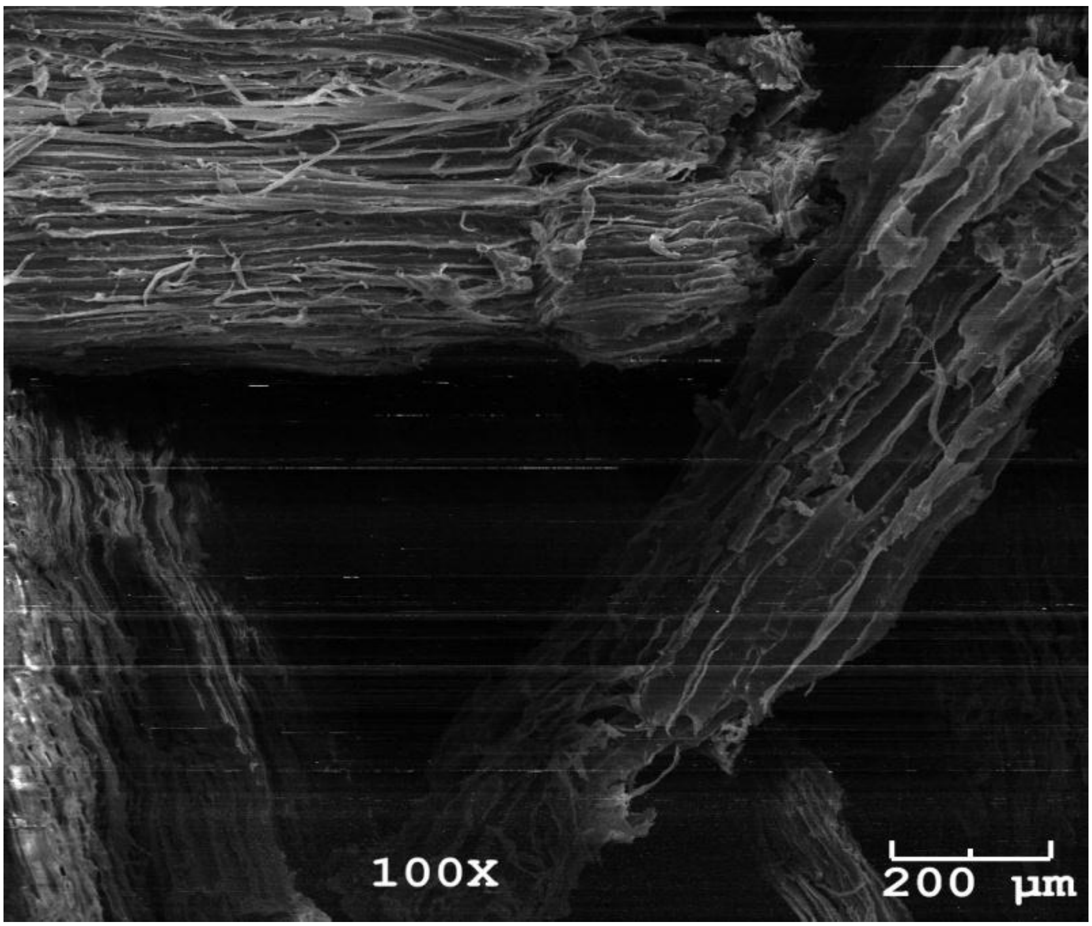

Figure 2. Biochar one residence time 160.7 sec with butane activity 6.25%.

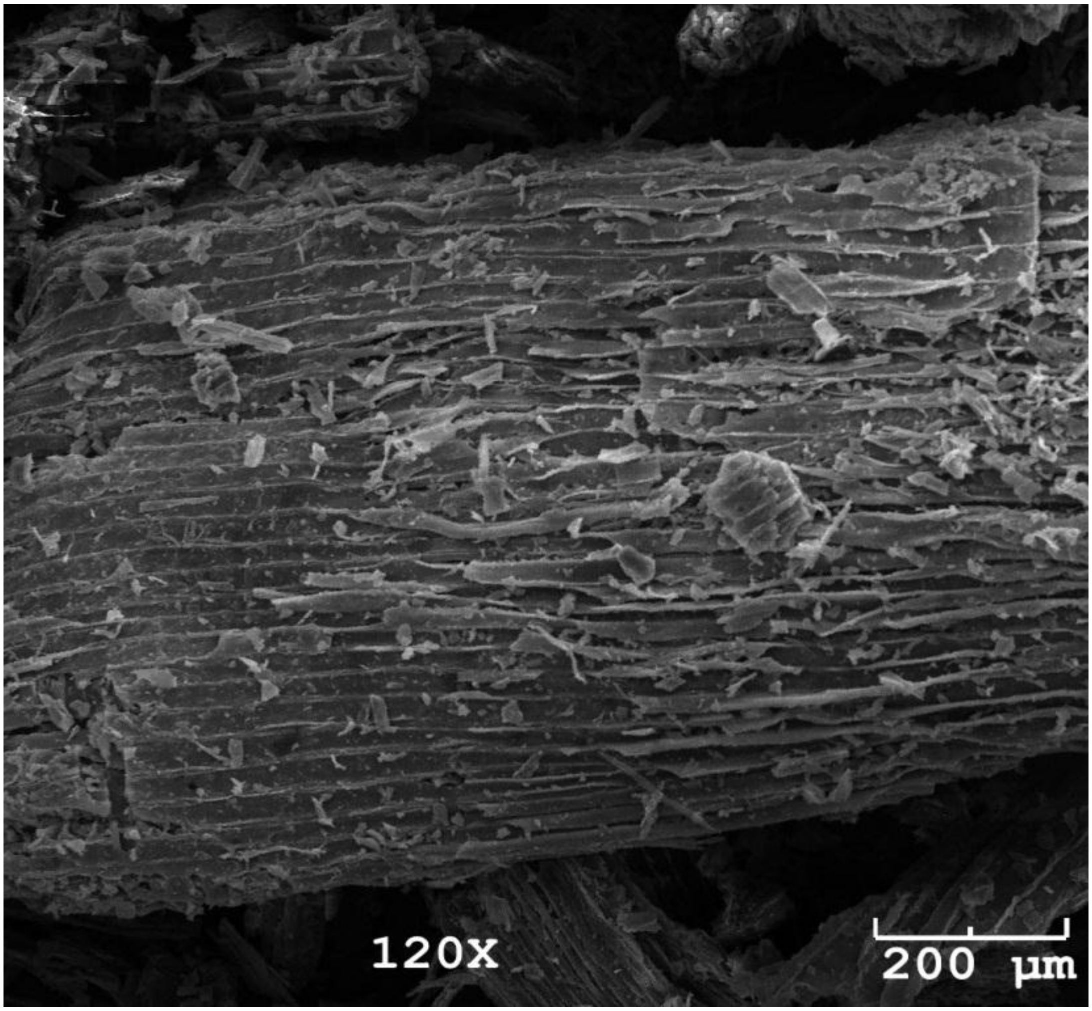

Figure 3. Biochar two residence time 134.4 sec with butane activity 5.5%.

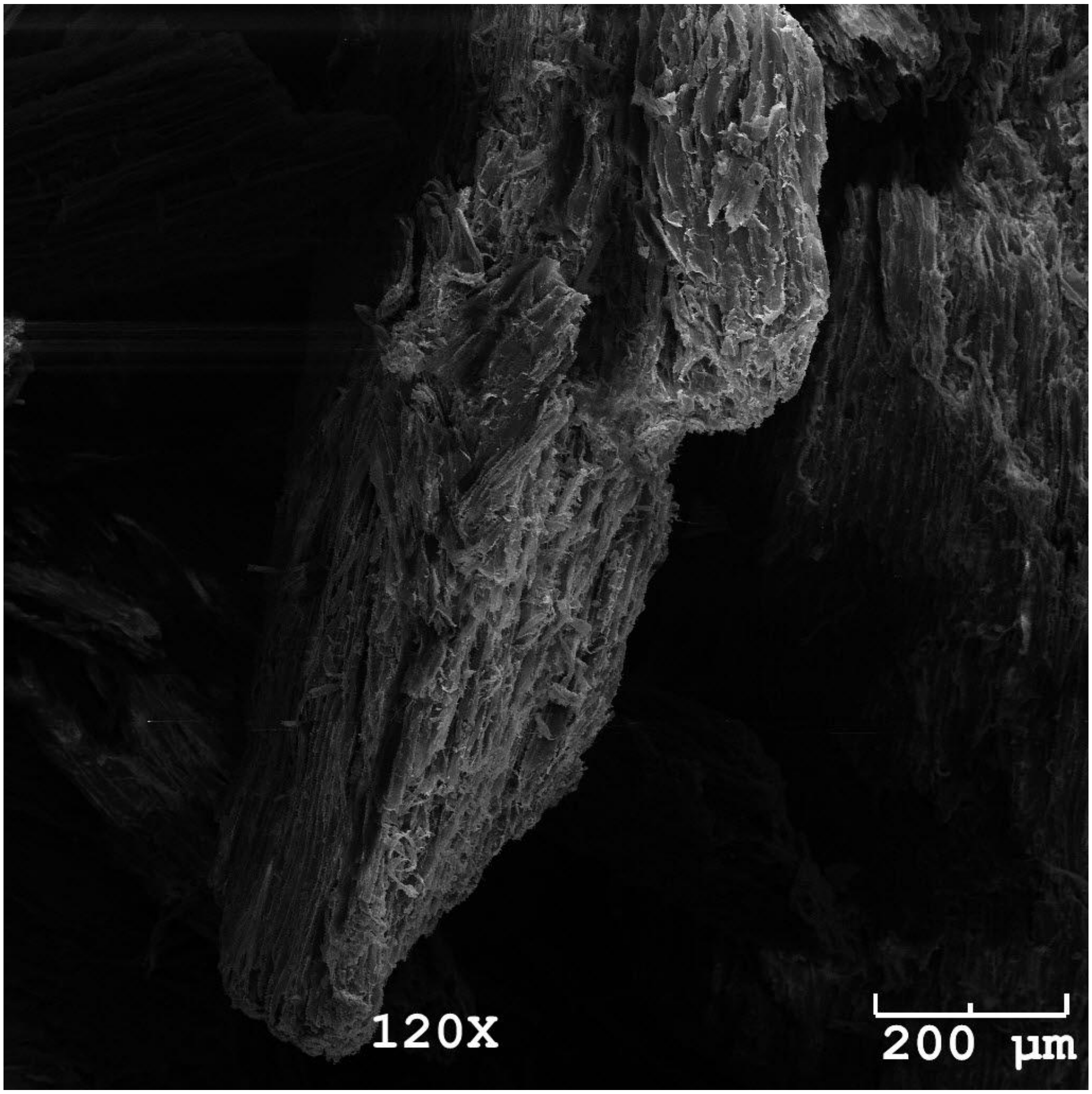

Figure 4. Biochar three residence time 97.3 sec with butane activity 2.35%.

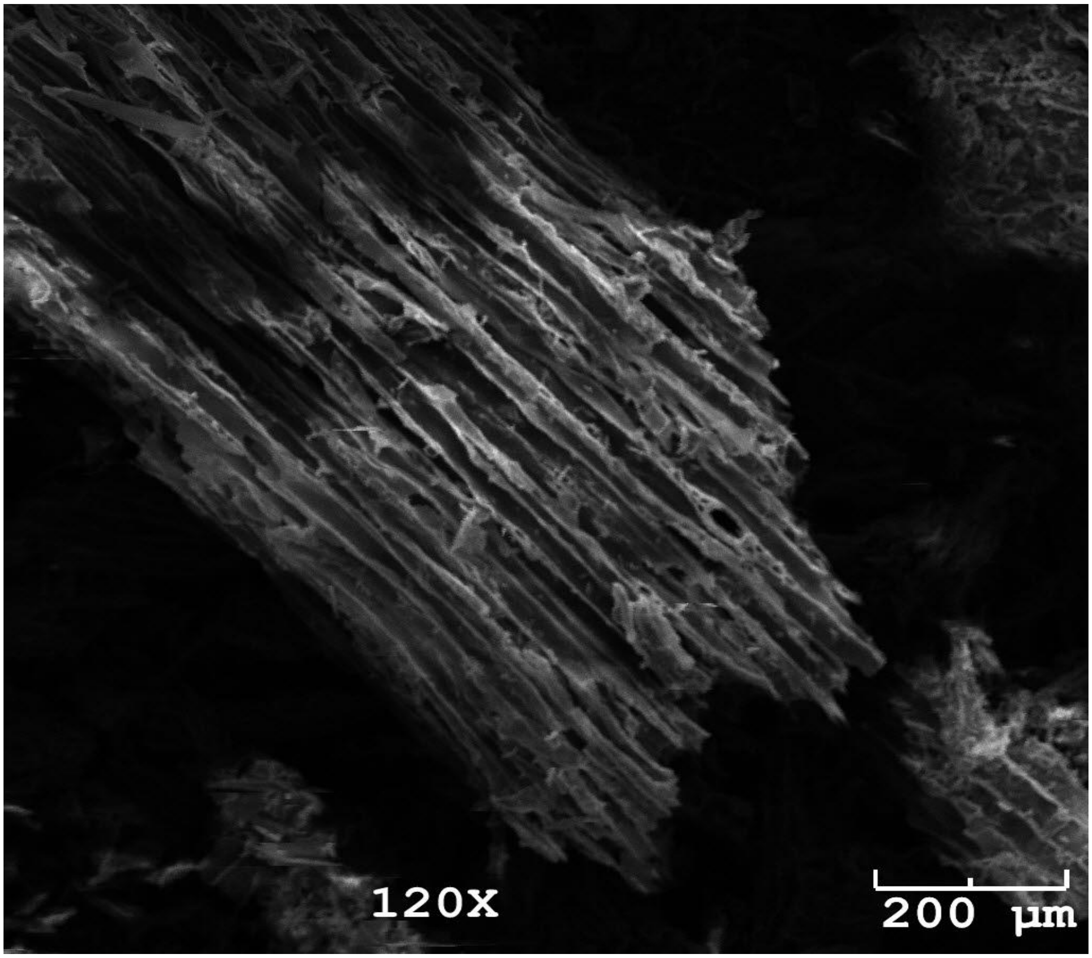

Figure 5. Biochar four residence time 81.97with butane activity 3.85%

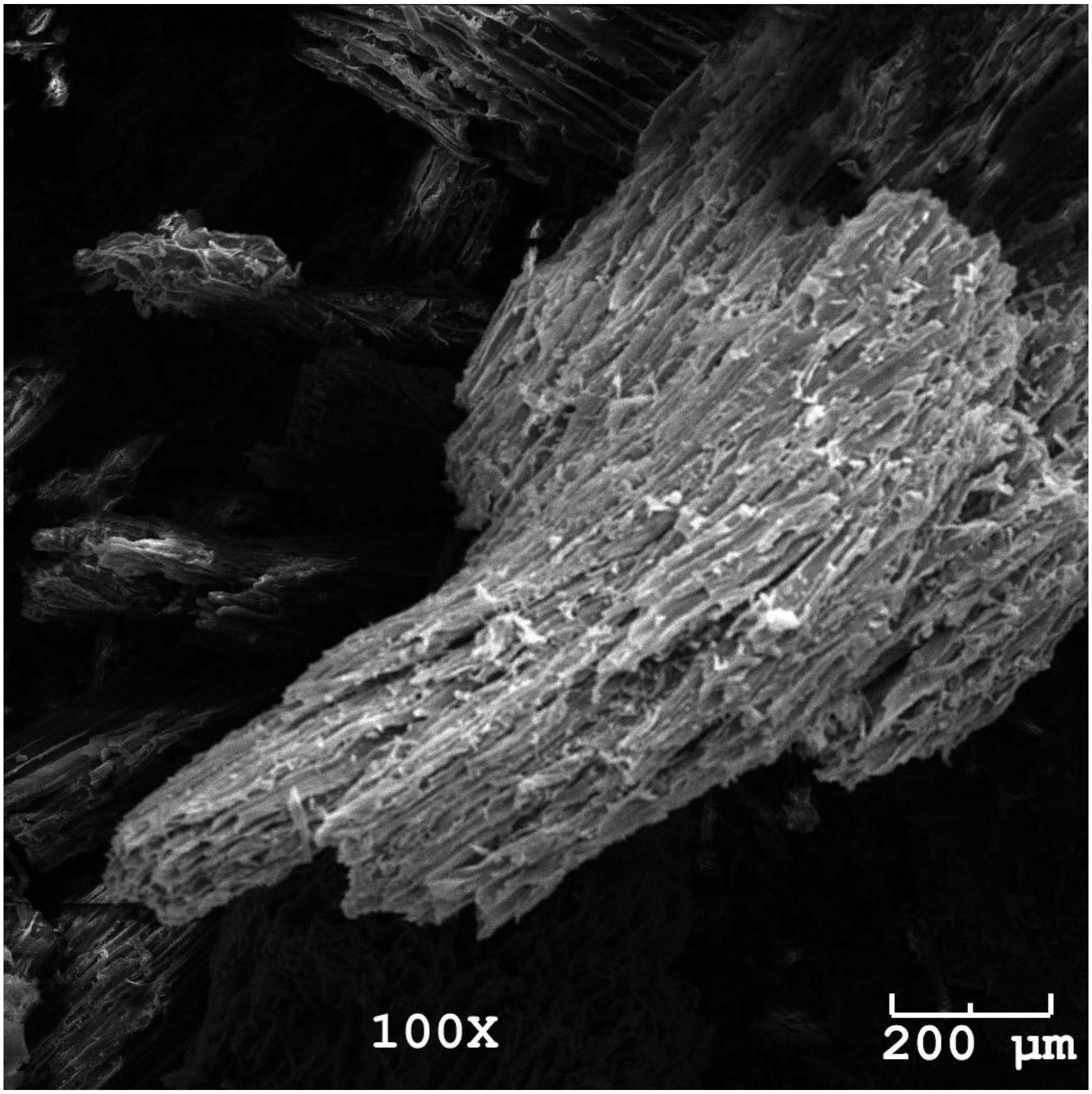

Figure 6. Biochar five residence time 73.65 with butane activity 8.8%

2.2.4 Soil analysis

The results of the chemical analyses of the two soil types are presented on Table 6. Chemical properties for cultivated and forest soils are different for each soil type.

Table 5. Chemical properties of the cultivated and forest soils used in this experiment

Soil type	**pH**	**EC dS/m**	**CEC cmol kg^{-1}**	**Total %C**	**Total %N**	**Total %H**
Forest	4.2	0.15	33.00	9.10	0.43	N/A
Cultivated	4.34	1.8	22.07	4.55	0.39	N/A

Available nutrients by Mehlich 3 extract and cation exchange capacity by 1NH4OAC is presented on Table7 and 8. Some nutrients like P, Na and Mn are significantly higher on cultivated soils. CEC is higher on forest soils than cultivated soils.

Table 6. Available nutrients by Mehlich 3 extract for cultivated and forest soils

Soil Type	**P (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Fe (mg kg^{-1})**
Forest	60	4.3	3.0	13.4	590
Cultivated	141	4.3	3.5	38.0	566

Table 7. Cations and exchange capacity by 1NH4OAC for forest and cultivated soils

Soil type	**K (cmol kg^{-1})**	**Ca (cmol kg^{-1})**	**Mg (cmol Kg^{-1})**	**Na (cmol Kg^{-1})**	**CEC (cmol Kg^{-1})**
Forest	0.30	9.70	2.39	0.03	33.00
Cultivated	0.17	9.55	0.98	0.13	22.07

2.2.5 Microbial activity: response surface model results for microbial activity response

2.2.5.1 Cultivated soils before freeze-thaw

The microbial respiration measured during the six days was used as the response for the response surface model. The ANOVA for the response surface quadratic model is significant with p = 0.003. The model illustrated in figure 2 shows a trend of high microbial activity when biochar amounts are high. For instance biochar 5 which has the shortest residence time of 73.5 sec shows high microbial activity at increased biochar rate. Similar trend was observed with biochar 2 with residence time of 134.4 seconds which exhibit high microbial activity specifically at 10 g of biochar. On the other hand microbial activity diminished with decreased biochar application rates for all biochars.

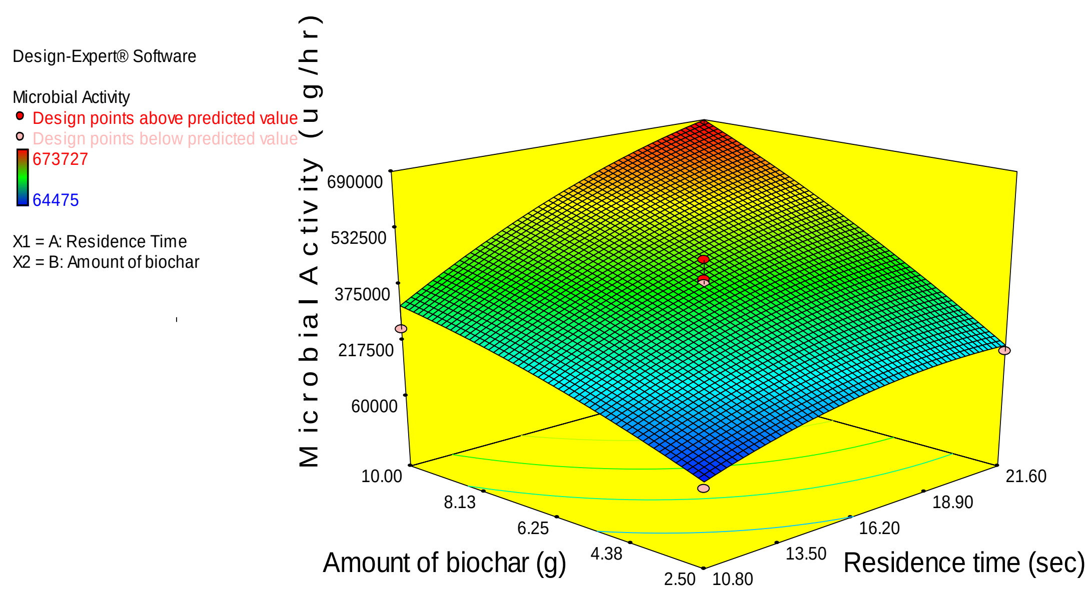

Figure 7. Microbial activity before freeze-thaw on cultivated soils

2.2.5.2 Cultivated soils after freeze-thaw

The ANOVA for the response surface quadratic model is significant with p = 0.001. Microbial activity decreased significantly after freeze-thaw. We can observe in Figure 1 that the highest range was 690000 ug/hr and in figure two the highest range 310000 ug/hr showing a reduction of respiration of 50 %. This reduction is wide-ranging for all biochar: soil ratios used in this experiment.

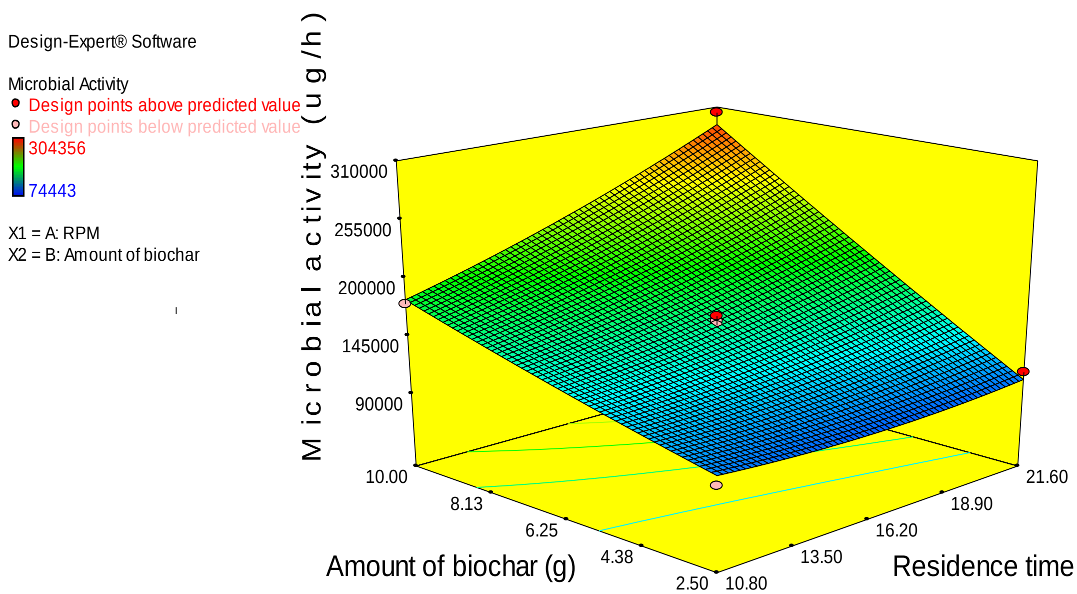

Figure 8. Microbial activity after freeze-thaw on cultivated soils

2.2.5.3 Forest soil before freeze-thaw

Microbial activity was significantly lower for forest soils compared to cultivated soils. The ANOVA for the response surface quadratic model was not significant with p =

0.2761. Figure 4 shows the very low activity exhibited by microbes for all type of biochars and amounts.

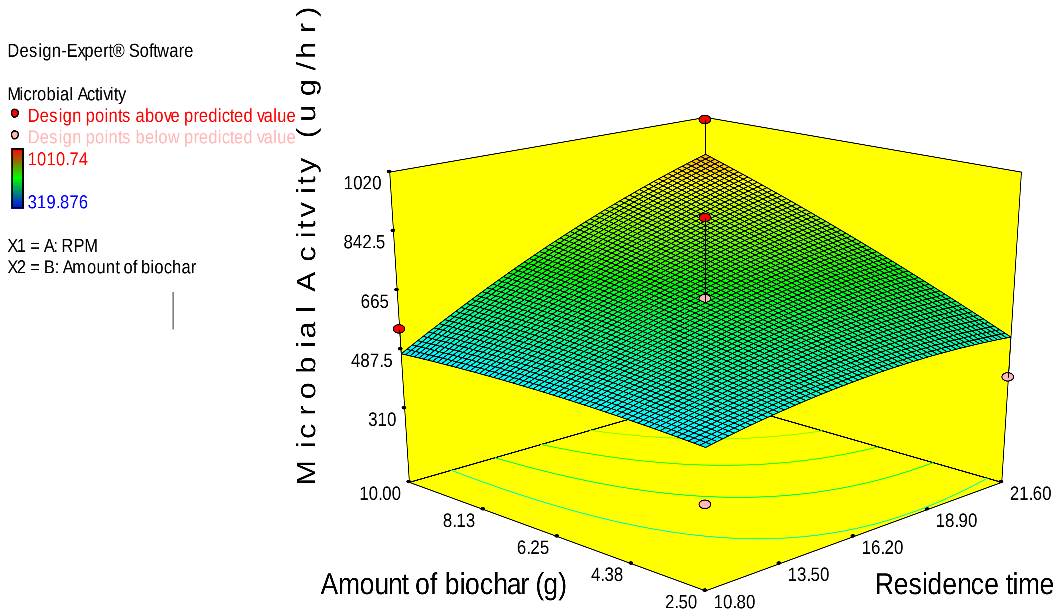

Figure 9. Microbial activity before freeze-thaw on forest soils

2.2.5.4 Forest soil after freeze-thaw

The ANOVA for the response surface quadratic model was not significant with p = 0.4708. We can observe in Figure 4 that microbial activity decreased to less than half after freeze-thaw as it occurred on cultivated soils, as shown in Figure 2.

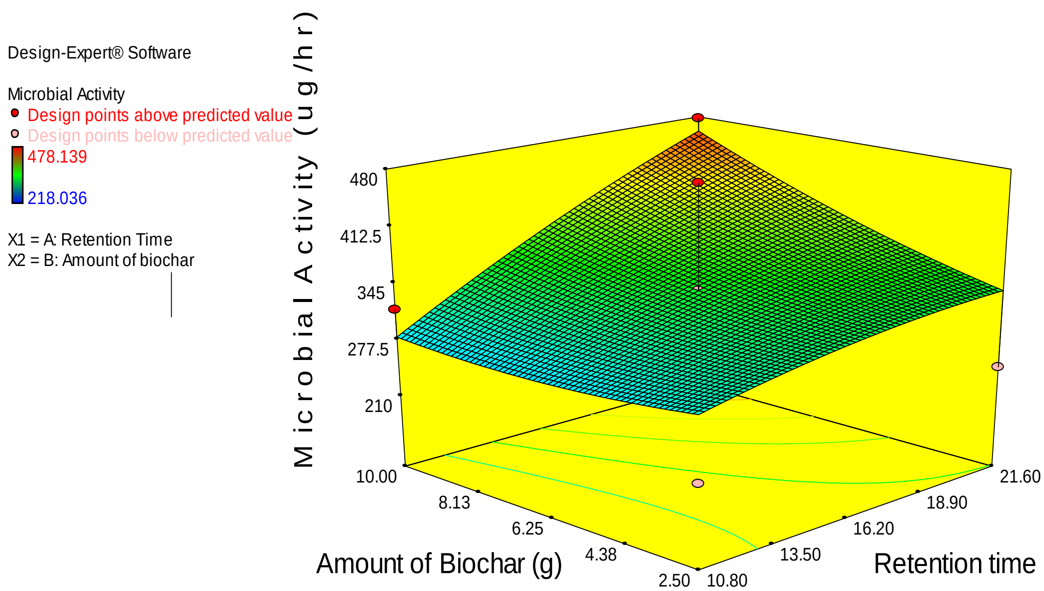

Figure 10. Microbial activity after freeze-thaw on forest soils

2.4 Discussion

2.4.1 Biochar chemical properties

Most chemical and physical properties of biochars were attained during pyrolysis (Lehmann, 2007). The residence time and temperature directly affect the chemical compositions of biochars specially related to pH, EC, CEC and surface area. In this study the results reflect the chemical changes on the different biochar types due to change on residence time.

The pH of the five different biochar types were acidic with the highest being 5.06 corresponding to biochar 2 with a residence time of 134.4 seconds, but pH in biochars can range between 4 to 12 depending on the feedstock (Lehmann and Joseph, 2009).

Cation exchange capacity was higher for biochars three, four and five which have the shortest residence time and it was lower for one and two which have the longest residence time. CEC is affected by temperature usually biochar at high temperature optimally between 450^{o}C and 550^{o}C seem to exhibit higher CEC. In this study temperature was held constant at 550^{o}C which has been described as an optimal temperature for biochar production (Lehmann, 2007). Total carbon was comparable for all five biochars, total nitrogen and hydrogen show higher values in the biochars with short residence time (three and four see table 3) where presumably H and N were not released during the gaseous phase because of the lack of heat exposure due to the short time spent in the heated area (Mašek et al., 2011). The biochars created for this experiment had some differences in nutrient availability; Biochar five for instance had high amounts of almost every element. Biochar three and four show significantly higher amounts of iron and manganese compared to others (see Table 4). We speculate that these results are affected by the residence time of this biochar. The shorter the time the biomass spends in the heated area where the transformation occurs, the less charred and mineralized it becomes. This specific biochar has a higher content of extractable nutrients due to less volatilization and mineralization occurring with the lower degree of charring (Bruun et al., 2011). There are some high amounts of manganese and Iron for Biochar three, with 10.8 ppm and 1018 ppm respectively, which we presumed to be caused by contamination from the pyrolysis unit. The same was assumed for Biochar four with regards to Mn and Fe. Though, Mn and Fe are preserved in large amounts during biochar formation in organic and inorganic forms (Lehman and Joseph, 2009).

All biochars show lignin bands similar to those mentioned on lignin- derived FTIR spectra (Sharma et al., 2004) and have similar functional groups. Table 5 shows the function groups of the five biochars and the original biomass sample. Figure 1 illustrates the FTIR spectra of the biochars and original sample respectively. FTIR analysis for all the biochars showed similar aliphatic and aromatic bands amongst them. We suggest that the similarity of these functional groups is due to the constant temperature of 550C^{o} maintained for all biochar and not enough differences in residence time to elicit a significant change in the functional groups present in the char molecules. It has been observed in other studies that lignin based biochars experience higher degrees of condensation and aromaticity when the charring temperature increases (Kim et al., 2012). The butane method helps to give an indication of the micropore volume of activated charcoal but it is a reliable method for observing trends on biochar adsorption capacity. Table 6 shows biochar one, two and five as having the larger butane percentage activity 6.25 and 5.5 respectively. Biochar one and two have longer residence and presumably less elastic biomass structure and contains a more graphite like structure which results in higher adsorption capacity (McLaughlin et al., 2012). In contrast biochars three and four have a less butane activity 2.35 and 3.85 percent indicating the potential presence of elastic biomass that have a less absorptive capacity indicating the lower adsorption capacity. Biochar five has the shortest residence time and showed an unexpected butane activity of 8.8 percent. The adsorptive capacity of this biochar was expected to be the lowest due the less degree of carbonization during pyrolysis. As described by

McLaughlin, 2012 residual water may have occupied butane sites affecting the butane adsorption capacity resulting in a higher adsorption activity.

2.4.2 Cultivated and forest soils properties

Undisturbed boreal forests have high content of organic matter due to the slow decomposition rates experienced in these regions. Organic matter is the main driver for some soils chemical properties to be enhanced in the absence of clay colloids. Soils with low organic matter such is the case of the cultivated soil used in this study present different chemical properties than forest soils.

As expected the undisturbed forest soils had the higher CEC, total carbon and nitrogen. Boreal forest soils have higher amounts of organic matter (Dixon et al., 1994) that contribute to high CEC, C and N. Both soils were acidic and electrical conductivity is higher on cultivated soils as a result of fertilizer application (Table 6). As could be expected, amounts of extractable nutrients for cultivated soils are higher for most nutrients measured (Table 7). The history of continuous fertilization and cropping of the cultivated site is likely responsible for the differences. Some elements like Fe, Cu, Zn, and K, were higher in the forest soil site. The forest soils have higher amounts of exchangeable bases than the cultivated soils except for Na, which is higher in cultivated soils due to the accumulation of salts because of the ongoing fertilization.

2.4.3 Biochar effect on cultivated soils before freeze-thaw

Samples with low amounts of biochar showed low microbial activity, indicating that microbial activity is higher in treatments that received higher amounts of biochar.

The highest microbial activity occurred during the first hours and slowly decreased in the course of the six days for all samples (see Appendix pg.49). The same reaction has been observed in other studies suggesting that "young biochars" have immediate sources of labile carbon for soil microbes (Smith et al., 2010) increasing respiration rates. The biochars were not washed prior to incubation suggesting that the presence of salts and tars were also available for microbial consumption, explaining the early high microbial activity. This correlates to studies in which biochar was washed before application which showed decreased CO_2 release by 50 percent compared to unwashed biochar (Jones et al., 2011). Samples with Biochar four and five showed higher activities compared to other biochar types that contained the same amount. These two biochars had the shortest residence times (81.97 and 71.93 seconds), and we presumed that at this residence time larger amounts of aliphatic and aromatic surface groups were accessible, resulting in increased microbial oxidation (Cheng et al., 2006). The amount of biochar has been described to affect microbial activity significantly when increasing biochar rates on temperate soils, especially those with low carbon content (Kolb et al., 2009). We can observe the same trend on cultivated soils of this study where an increase of biochar increased microbial activity.

2.4.4 Biochar effect on cultivated soils after freeze-thaw

Results illustrated on Figure 2 clearly shows that microbial activity decreased by almost half compared to the microbial activity measured before freezing(see Appendix pg.49), as shown in Figure 1. This reduction in activity is consistent with results of other studies in which microbial populations were reduced up to 50 percent in a single freeze cycle (Soulides and Allison, 1961) and the same decrease is shown in control sample (see Appendix pg.50). Once again biochar with the shortest residence time exhibited higher microbial activity than others. In the same way as it occurred before freeze-thaw the highest microbial activity took place at higher biochar rates 6.25 g, 10 g, and 11.25 g for all biochars. This same trend has been observed in other studies supporting that at high biochar rates there is higher microbial respiration (Kolb et al., 2009). A high respiratory burst of CO_2 was detected in measurements when samples thawed. However the average microbial respiration was recorded after the initial CO_2 burst that occurs during thawing (Skogland et al., 1988; Soulides and Allison, 1961) during the first hours. The lysed microbes likely provided large amount of dissolved sugars and amino acids to surviving microbes, causing the respiratory burst after thawing (Ivarson and Sowden, 1966; Schimel and Clein, 1996; Skogland et al., 1988).

2.4.5 Biochar effect on forest soils before freeze-thaw

The results of this model show how microbes on forest soils had very little response to biochar compared to cultivated soil results. The highest microbial activity was detected on the initial hours of measurements then slowly decreased (see Appendix pg.57).

Although the model does not indicate significant response to biochar, microbial activity was high at biochar amounts 6.25 g, 10g and 11.25 g respectively, showing the same trend as seen in cultivated soils that higher biochar rates increase microbial activity. Once again biochar five exhibit high microbial activities at a medium biochar rate 6.25 g and biochar four exhibit the highest activity at the highest biochar rate. Boreal forest soils contain large amounts of soil carbon (Dixon et al., 1994) suggesting that a large stable carbon pool exists on boreal forest soils, and the addition of biochar does not have a significant effect on microbial activity. Temperate soils with higher C showed lower metabolic quotient than soils with lower C at increased charcoal rates (Kolb et al., 2009) emphasizing that biochar can provide more benefits to soils with lower carbon such as low organic matter content, low pH, excessive drainage and other properties that decrease soil quality. The presence of recalcitrant C in biochar high latitudes can also benefit the increase of N in soils due to the mining of recalcitrant C for N by soil microbes (Craine et al., 2007).

2.4.6 Biochar effect on forest soils after freeze-thaw

Results indicated that microbial activity decreased to less than half after freeze-thaw, which is in agreement with other studies showing that microbial activity can decrease to almost half in a single freeze-thaw cycle (Soulides and Allison, 1961). The same occurred on cultivated soils, as shown in (Figure 2) reiterating how soil microbial population significantly decreases after freeze-thaw. As was observed in cultivated soils microbial activity was also the highest during thaw and then slowly decreased for forest soils (see Appendix pg.62). Figure 4 shows the same trend observed in the other models larger rates of biochar application, where 6.25 g, 10 g, and 11.25 g showed higher microbial respiration than at lower biochar rates of 0.95 g, 2 g. The highest microbial activity in this model occurred during thawing due to the respiratory burst explained above.

2.4.7 Conclusion

The results of this study indicated that cultivated soils of subarctic soils of sub-central Alaska can get more benefit from biochar additions than boreal forest soils, in term of stimulating microbial activity. Producing biochars with complete conversion of the biomass into biochar is important for the long-term stability of the product in soil. The effect of freeze-thaw and the influence of biochar on microbial activity in this experiment led to the observation that microbes suffer the same detrimental population loss with biochar additions as in soils not amended with biochar, as seen in other studies. Nonetheless, Biochar can provide great benefit to cultivated subarctic soils during periods free of freeze-thaw. Biochar could benefit soil microbial activity and other soil properties that in turn could provide a benefit to cultivated subarctic soils that are low in carbon. Cultivated subarctic soils responded more positively to biochar amendments at higher application rates than lower ones. Microbial activity and biomass contribute greatly to the carbon cycle and nutrient turnover that in turn may benefit above ground biomass and production.

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Chapter 3 Conclusion

The addition of biochar to cultivated and forest soils in this study demonstrates how differently it can affect microbial activity depending on application rate and natural soil properties. In all treatments, biochar proved to be more efficient in cultivated soils than in forest soils. The amount of organic carbon present in the soil provides the primary source of carbon and energy for soil microbes and the amounts available in a specific soil type will determine the need for biochar inputs. Cultivated soils in this study had the lowest amount of organic carbon and gained the most benefit from biochar additions as shown in Chapter 2. On the other hand, forest soils showed an insignificant response to biochar additions in this model presumably because of the higher, natural levels of organic carbon in this soil type. In forest soils, organic matter is high and there are different organic substances that contain C (Brady and Weil 2008) including labile and recalcitrant organic matter. In a soil that is carbon rich it is assumed that the soil carbon storage gets replenished with standing vegetation added to the soil as plant litter. Further this plant litter decomposes and gets metabolized by organisms that produce stable organic compounds that will remain in the soils for a long time, just as biochar does. In conclusion a carbon rich soil may not experience the advantages of adding biochar because a stable carbon pool already exists.

Alaska large scale agriculture is slowly growing but home vegetable gardens have gain popularity and the need of a low cost local amendment may be appealing for farmers and gardeners. This study confirmed the positive effects of biochar at the biological level by showing that biochar boosted microbial activity even when added in small amounts (0.95 g and 2.5 g) in cultivated soil with low organic carbon. The soils available to farming in the state are young soils with low fertility that lose large amount of carbon after conversion, especially when sources of nitrogen are present either as fertilizer or atmospheric (Grünzweig et al., 2003). The addition of biochar to fields can help replenish some of the carbon in a recalcitrant and labile form that will last and benefit the soil for long periods because its stability in the environment is valuable for carbon storage (Singh et al., 2012). In conclusion biochar addition can greatly increase the carbon content and have direct effects on microbial communities that contribute to the turnover of nutrients necessary for plant growth.

Finally the effects of freeze-thaw on microbial activity with biochar additions demonstrated that soil microbes suffer the same detrimental reduction in population after freeze-thaw cycles with or without biochar additions. Our hypothesis that larger surface areas could provide larger amounts of unfrozen-water for soil microbes to thrive was not supported by our results. Instead microbial activity decreased to half after the freeze-thaw cycle and the same results were observed on the forest soils. These results are in agreement with other studies on the freeze-thaw effect on soil microbes.

The present production of biochar is still expensive and the technology needed for conversion can be sophisticated, especially for large-scale production. Traditional charcoal production is not suitable for making biochar since it is inefficient and creates pollution, and also the process is unable to control important biochar properties (Brown, 2011). Depending on the source of feedstock, the use of pyrolysis to create biochar requires some level of engineering since continuous feed pyrolysis units control several variables such as temperature, residence time, pollution emissions, feedstock moisture, yields and may not be simple for an individual to develop such a device (http://www.biochar-international.org/technology/production. By Brown Robert C. last accessed 2/13/2013). Nonetheless, smaller biochar production can be less complicated, inexpensive, and produce lower emissions, as is the case with biochar ovens made from simple materials. However, these ovens can only supply small batches of biochar; yielding about 12 kg in four hours, enough for small gardens. Biochar is a new growing technology that is becoming more and more popular around the world. Therefore, production and cost will become affordable as biochar demand increases.

Future works on Biochar in subarctic regions should include field trials that test plant response to biochar addition, nutrient retention, and adequate biochar application rates for subarctic soils. If microbial activity was enhanced by biochar additions in cultivated soils it is likely that biochar can ameliorate some soil fertility issues in subarctic regions. This can potentially increase decomposition and decrease organic matter content thus other management practices such residue management and no till may be necessary. Biochar is a soil enhancer that helps to adjust and improve natural soil properties in soils that have physical, chemical and biological limitations which is the case of subarctic soils. Over time benefits of applying biochar to these soils is but not limited to reduction of fertilizer use, irrigation, transportation and other amendments. If the soils develop to be healthy and productive with all the benefits that biochar can contribute at all levels along with correct practices we can say that the changes would likely be long lasting. The interactions between plants and biochar use have not sufficiently been studied in northern regions but there is substantial research and evidence on biochar effectiveness. It is hard to say how long the charcoal will remain in the soil and the benefits would persist, but charcoal with similar physical and chemical properties found in the Amazon have remained in the soil from pre-Columbian times to this day providing great benefit in highly weathered soils (Lehmann et al., 2003).

Thus, it is very important to continue working with biochar as soil amendments in these fast growing subarctic regions in order maintain good stewardship of agricultural soil.

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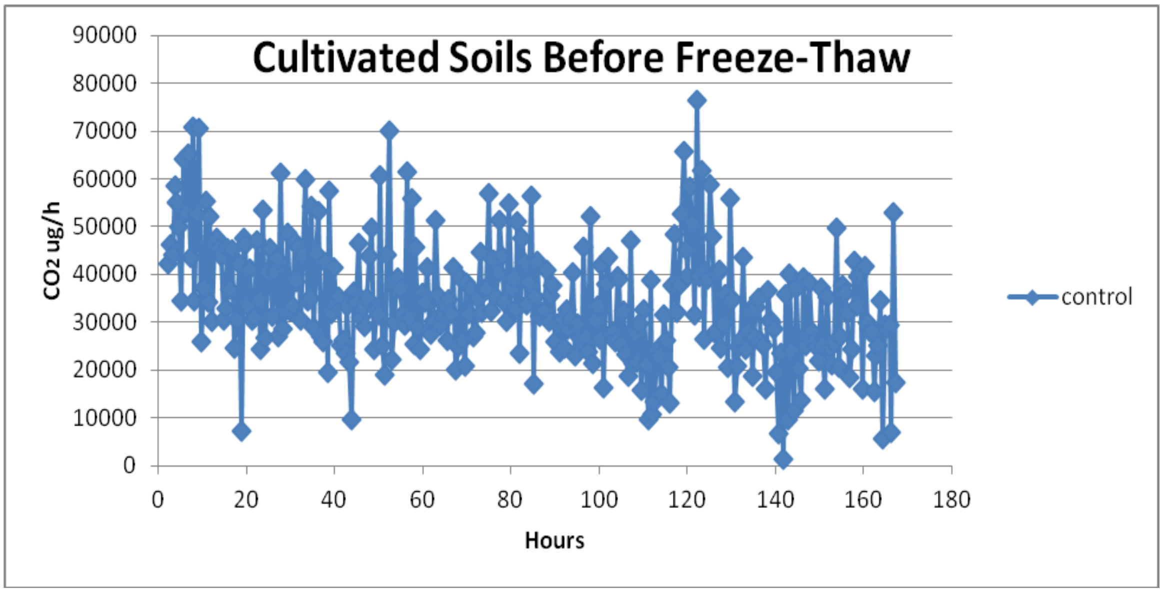
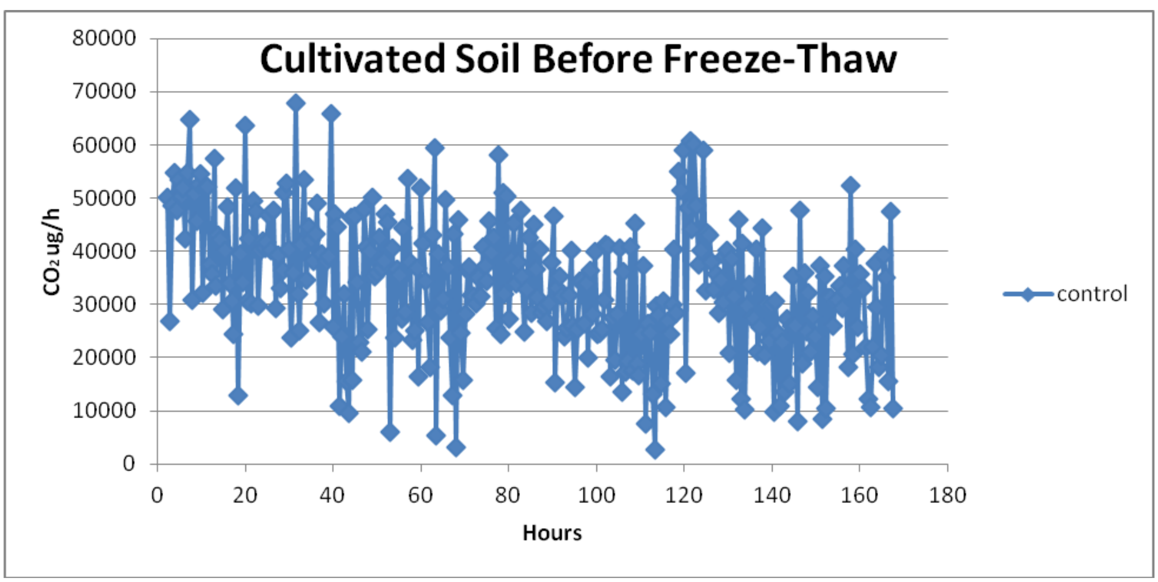
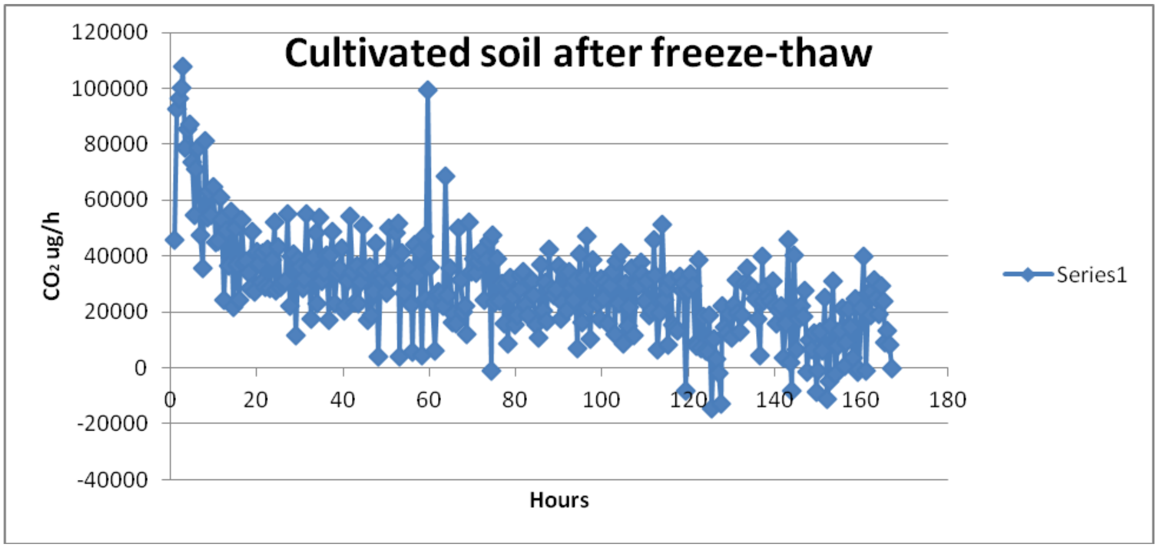
Appendix

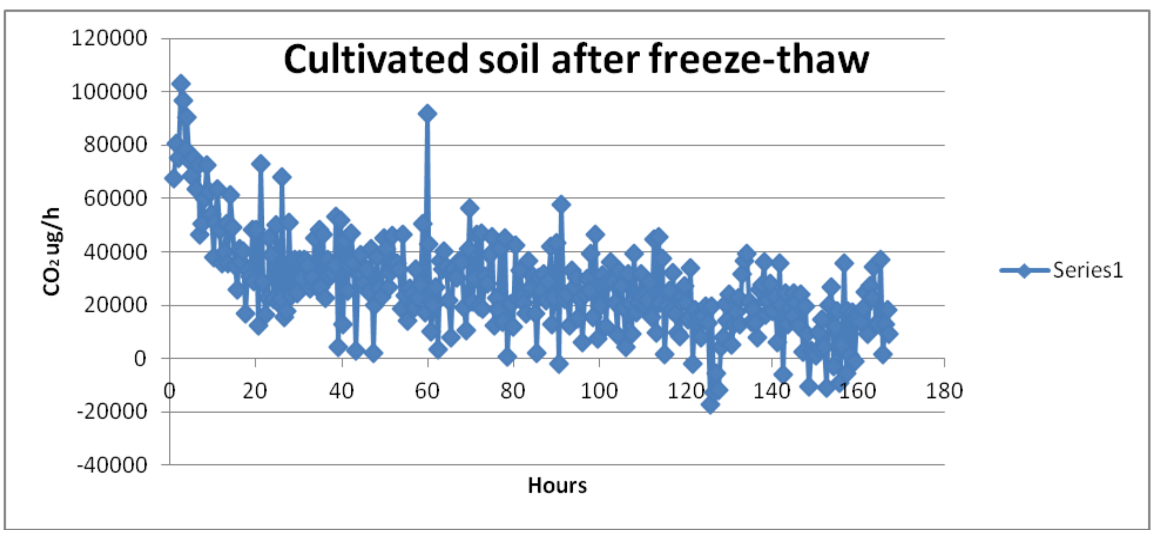
Control cultivated soil before-freeze thaw (two replicates)



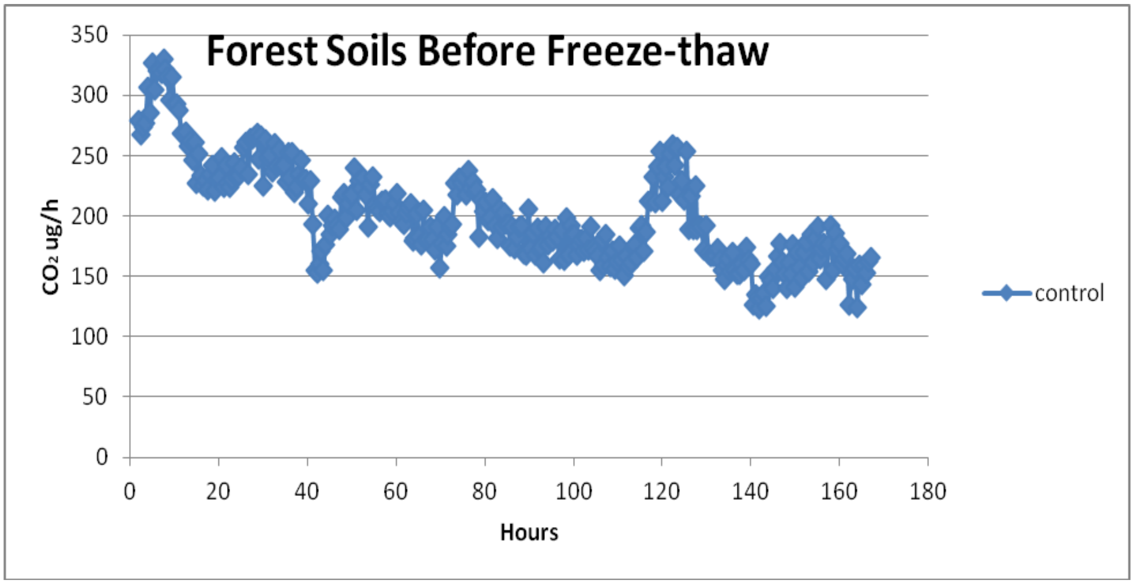


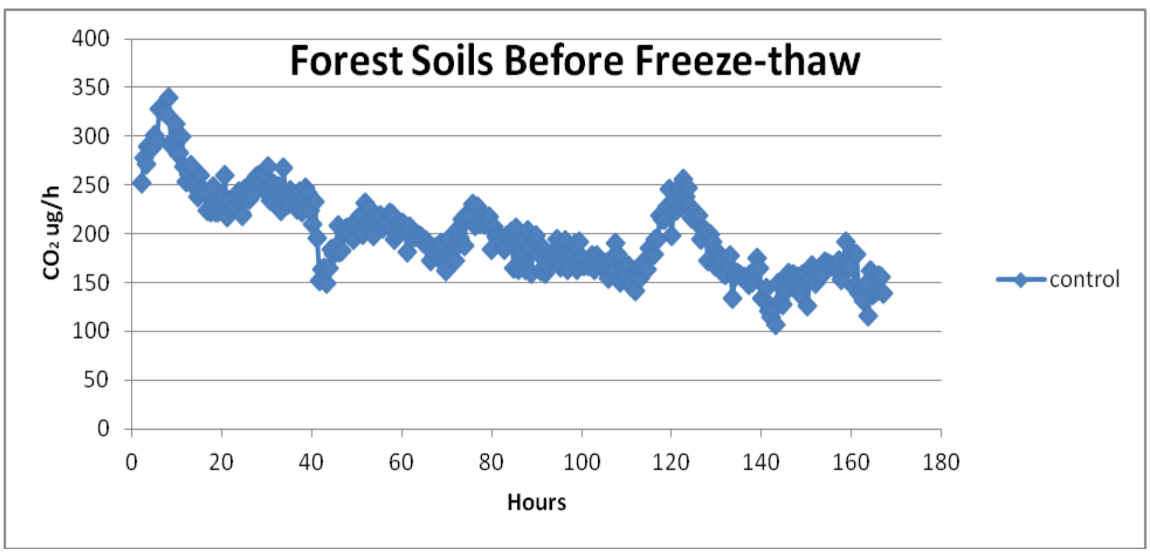
Control cultivated soils after freeze-thaw (two replicates)



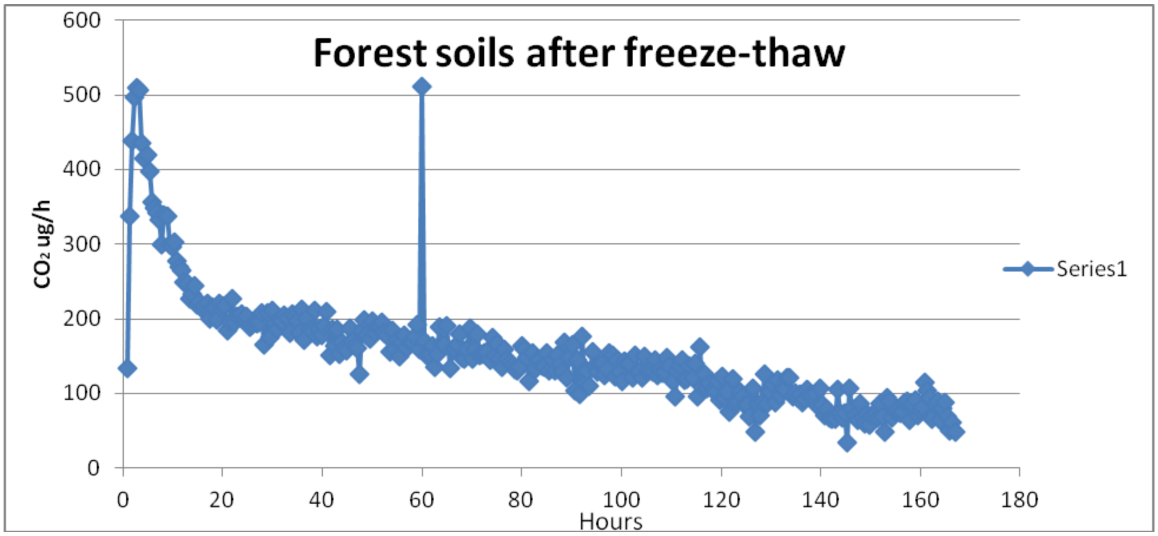


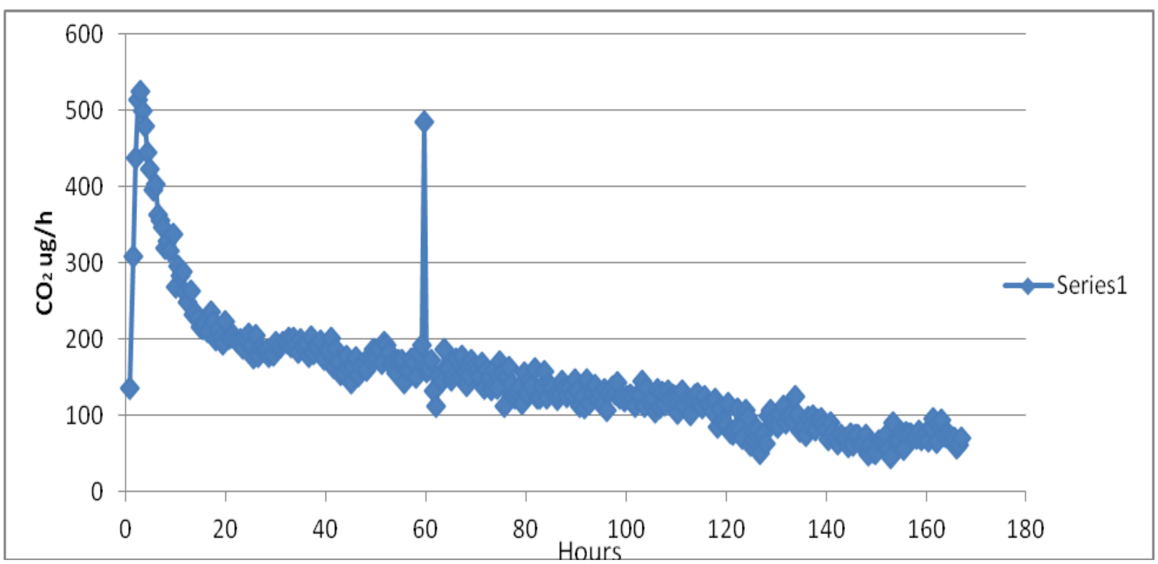
Control forest soils before freeze-thaw (two replicates)



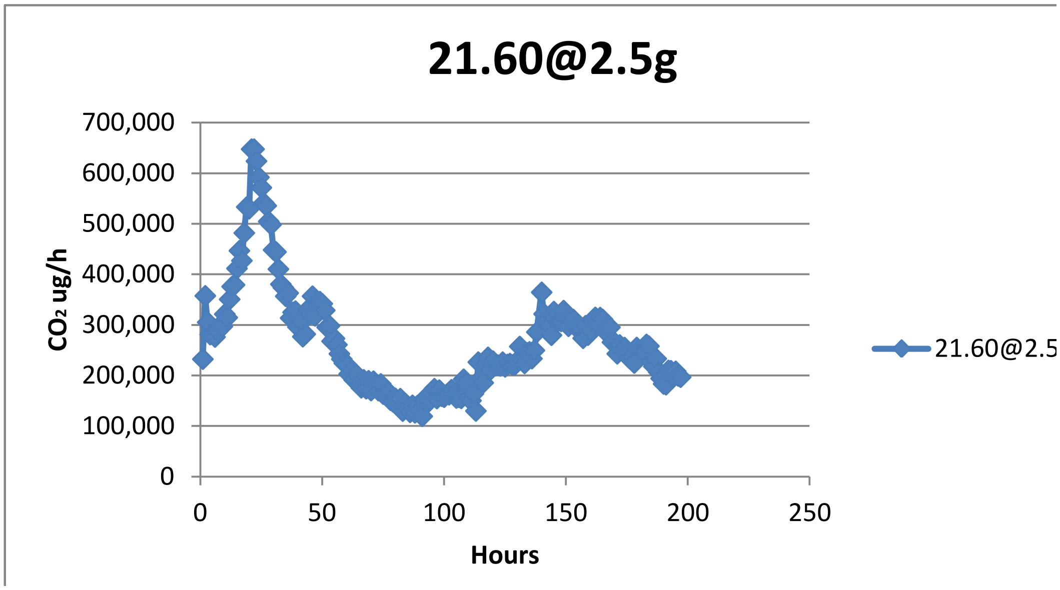


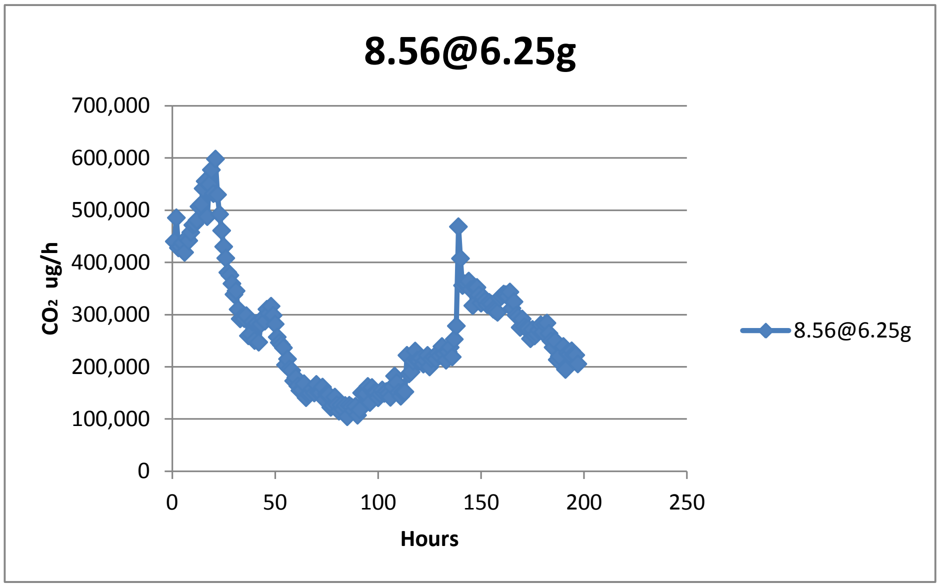
Control forest soils after freeze-thaw (two replicates)

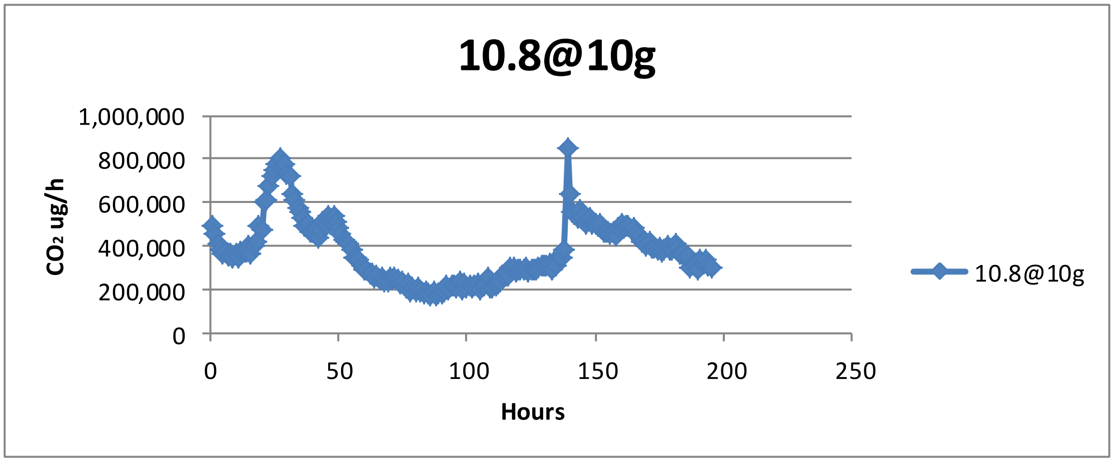


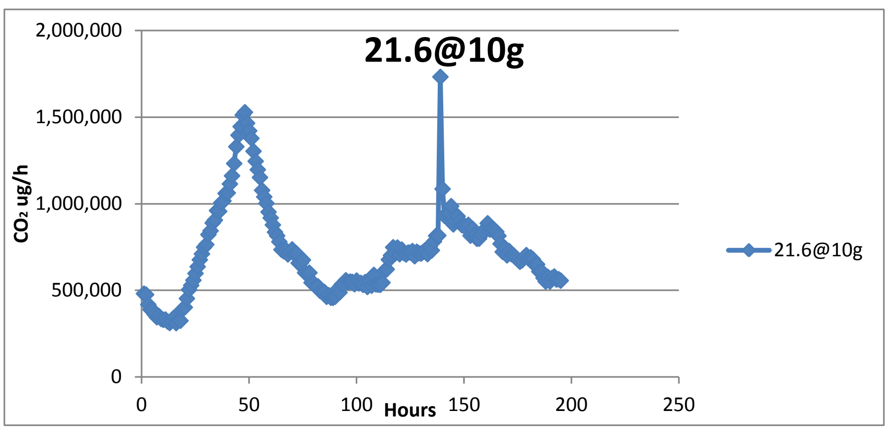


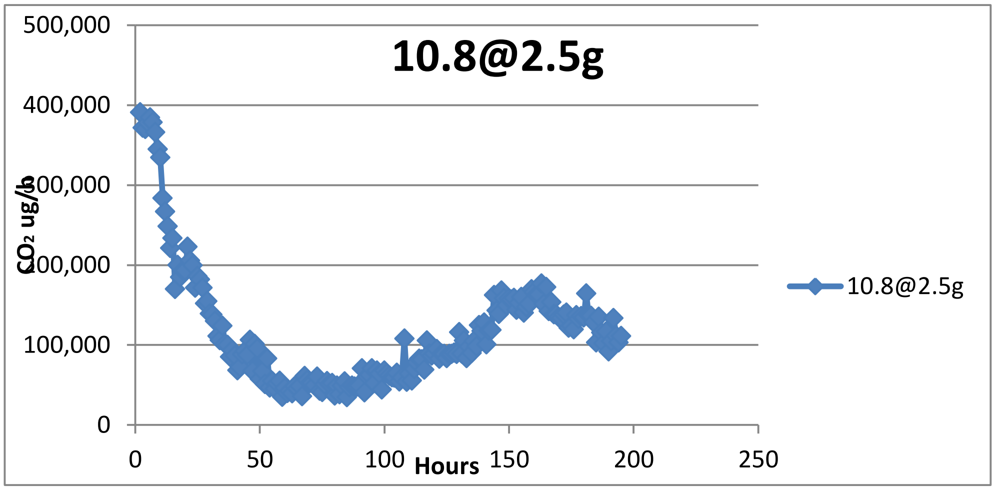
Cultivated soil before freeze-thaw with different biochar applications

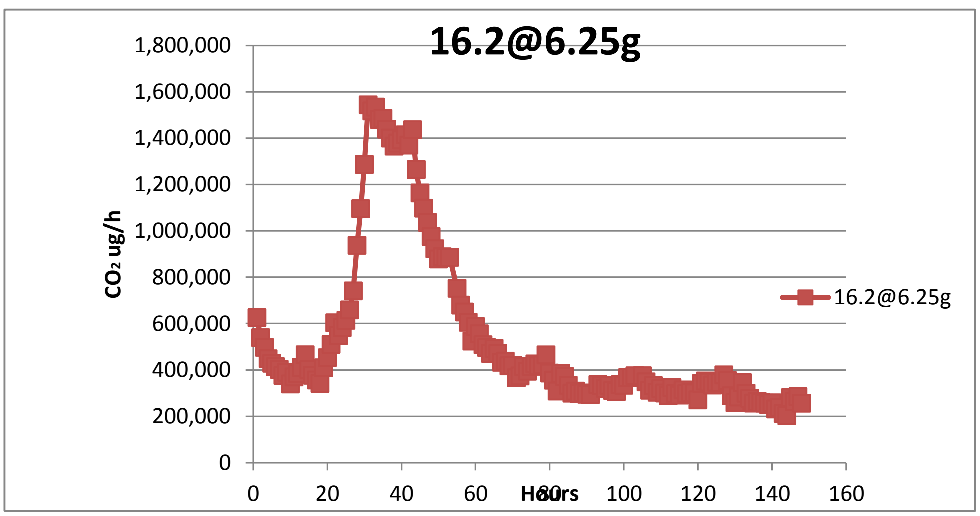


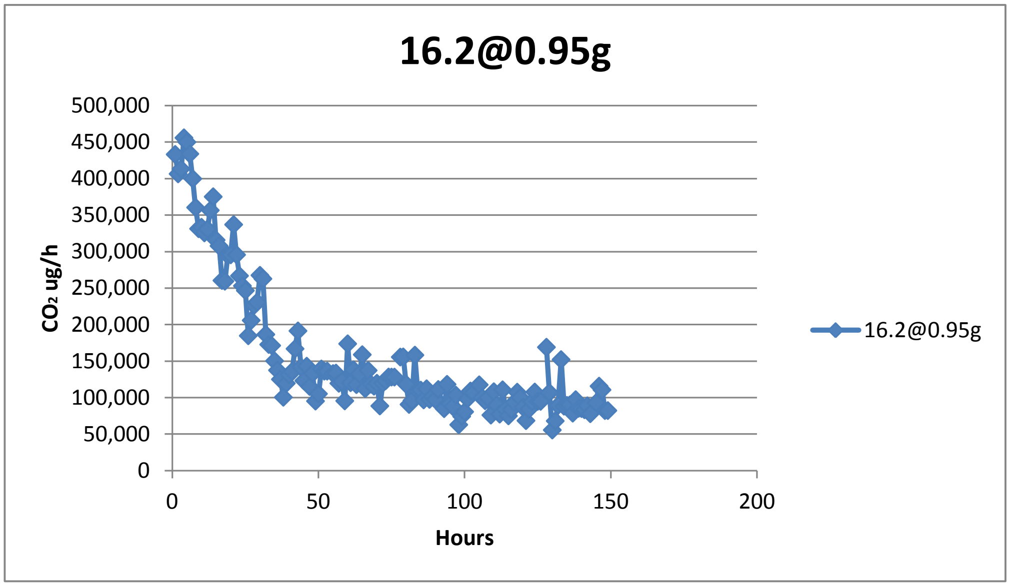



10.8@10g
1,000,000
800,000
600,000
400,000
200,000
0
CO₂ ug/h
0
50
100
150
200
250
Hours
10.8@10g

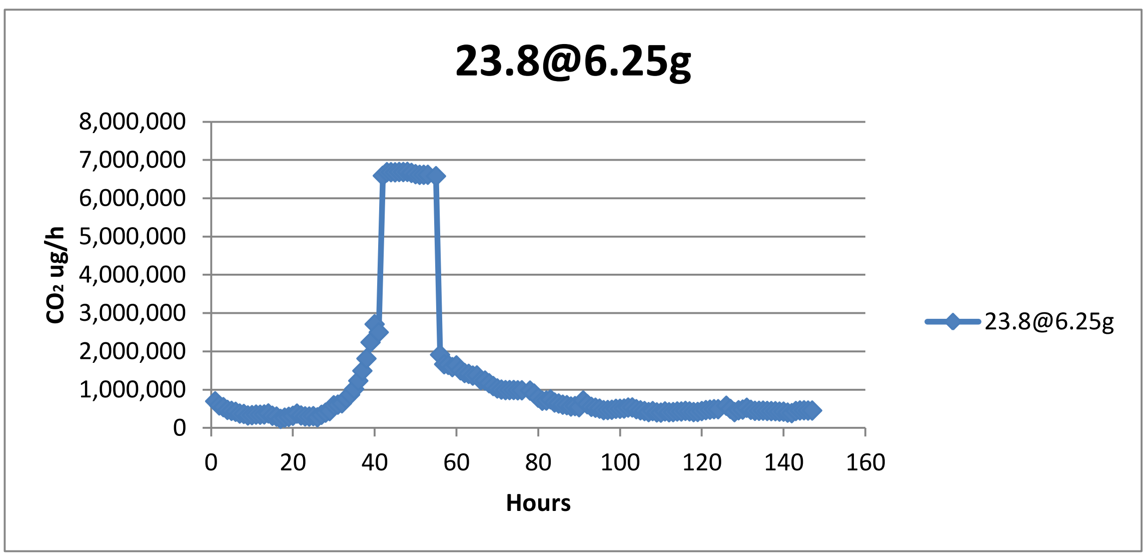

21.6@10g
2,000,000
1,500,000
1,000,000
500,000
0
CO₂ ug/h
0
50
100
150
200
250
Hours
21.6@10g


10.8@2.5g
500,000
400,000
300,000
200,000
100,000
0
CO2 ug/h
0
50
100
150
200
250
Hours
10.8@2.5g

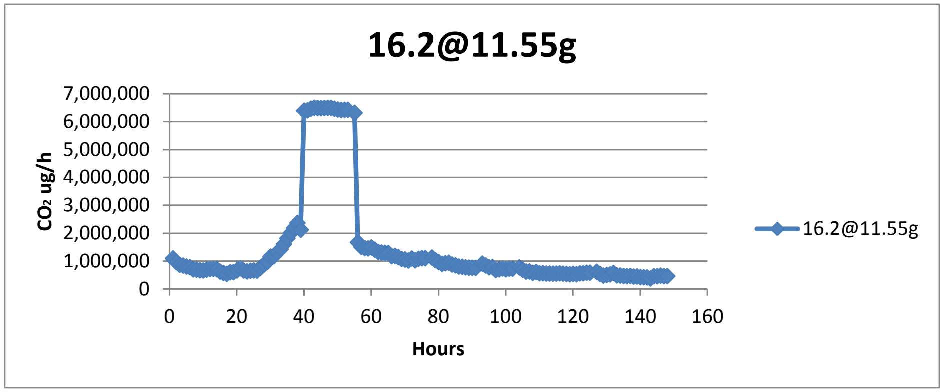

16.2@6.25g
1,800,000
1,600,000
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
CO2 ug/h
0
20
40
60
80
100
120
140
160
Hours
16.2@6.25g



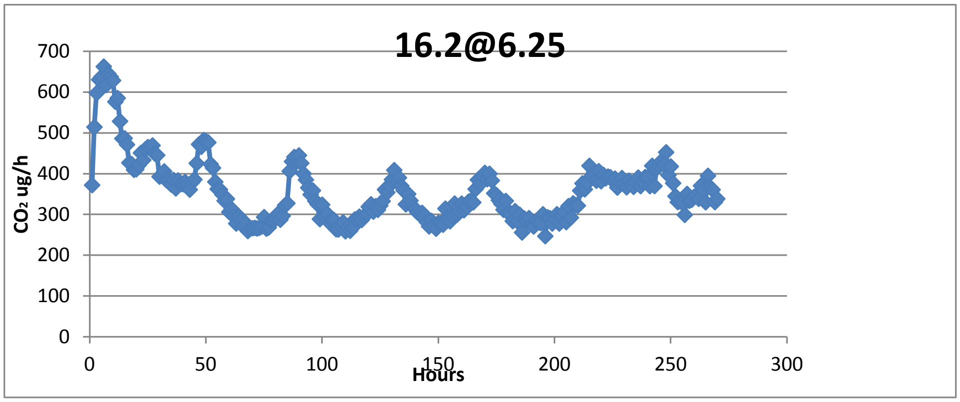
16.2@0.95g
500,000
450,000
400,000
350,000
300,000
250,000
200,000
150,000
100,000
50,000
0
CO2 ug/h
0
50
100
150
200
Hours
16.2@0.95g

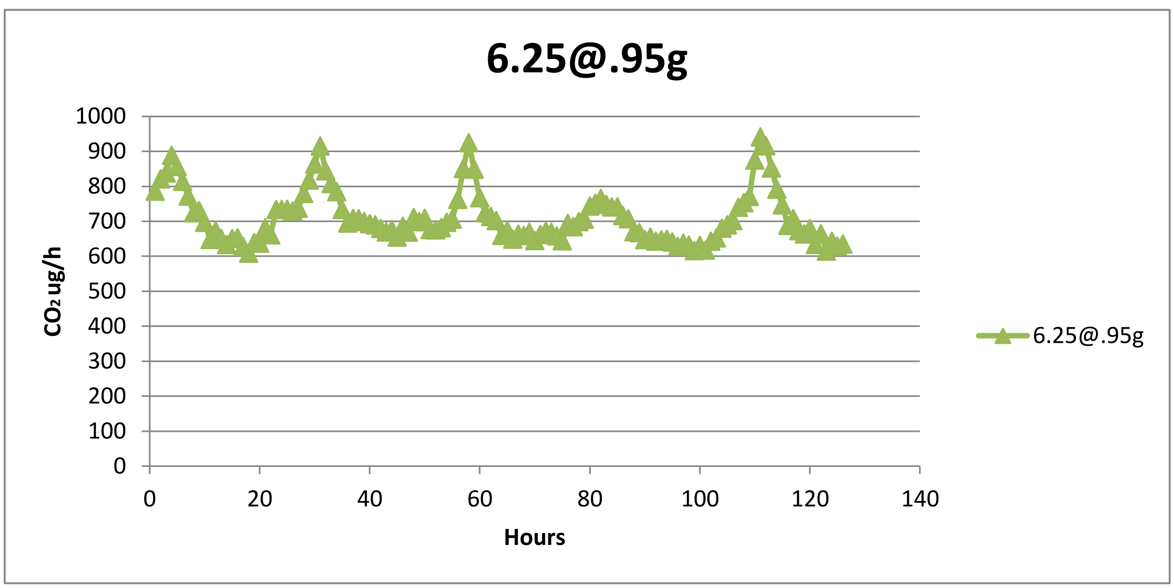


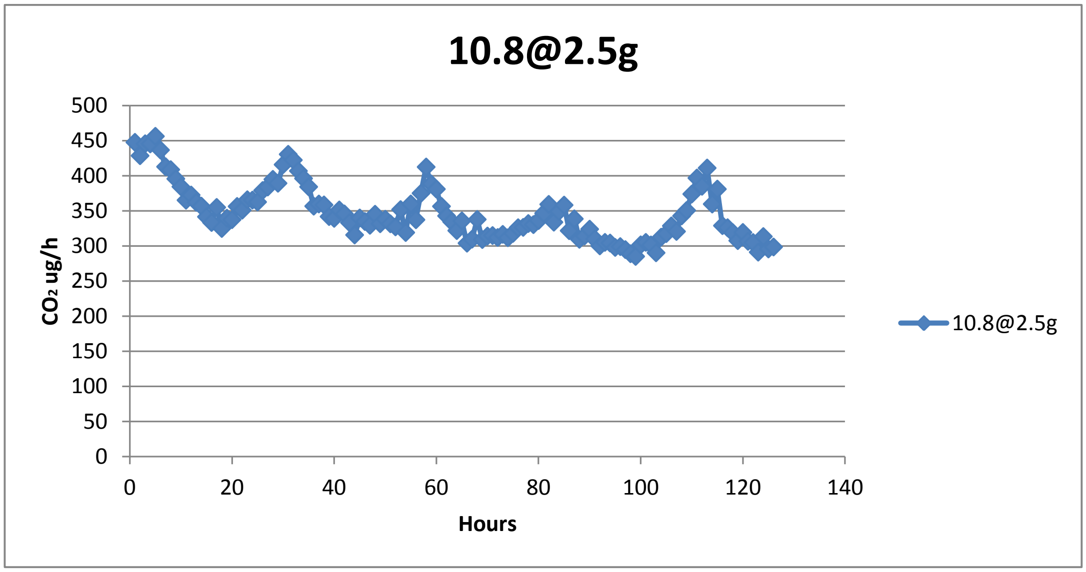
23.8@6.25g
8,000,000
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
CO2 ug/h
0
20
40
60
80
100
120
140
160
Hours
23.8@6.25g

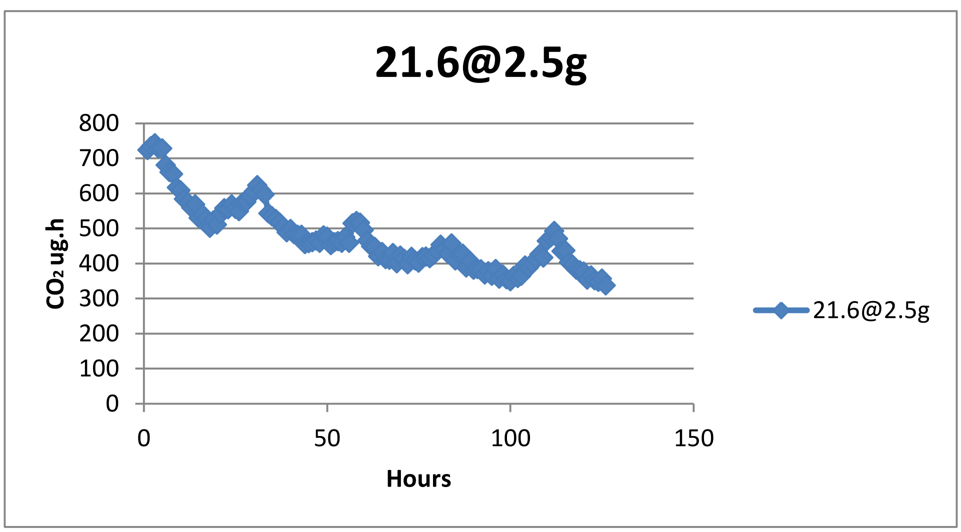

16.2@11.55g
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
CO2 ug/h
0
20
40
60
80
100
120
140
160
Hours
16.2@11.55g

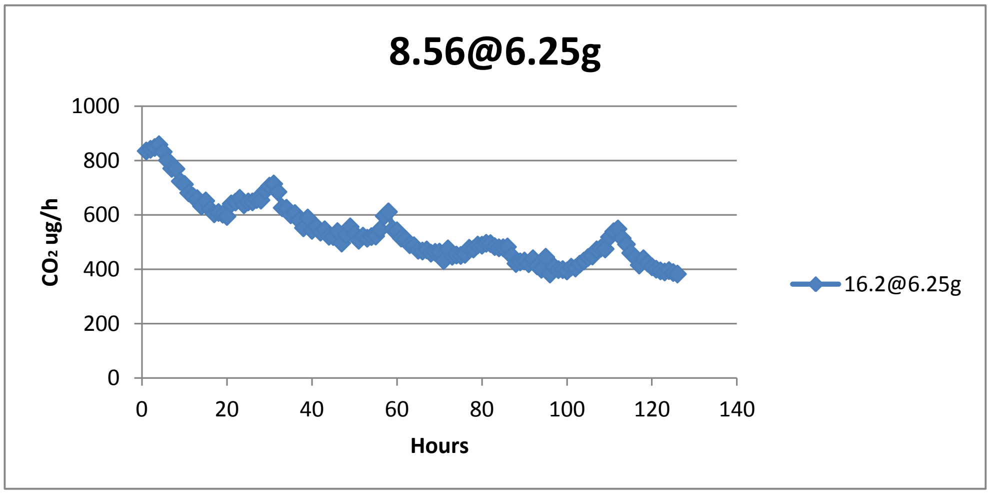
Forest soil before freeze-thaw

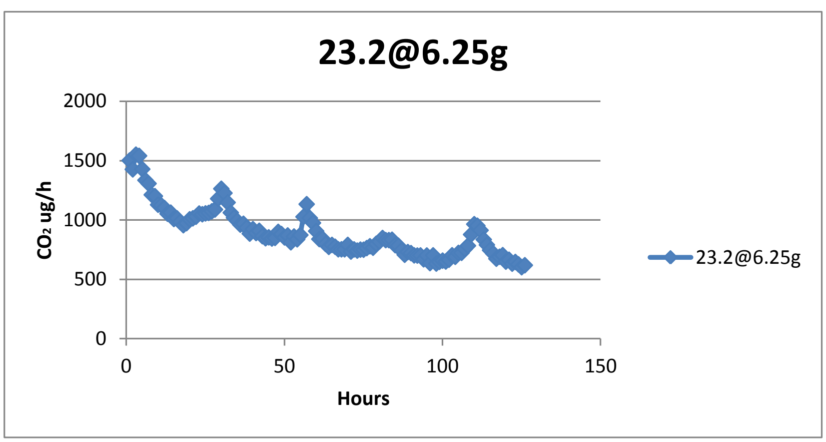


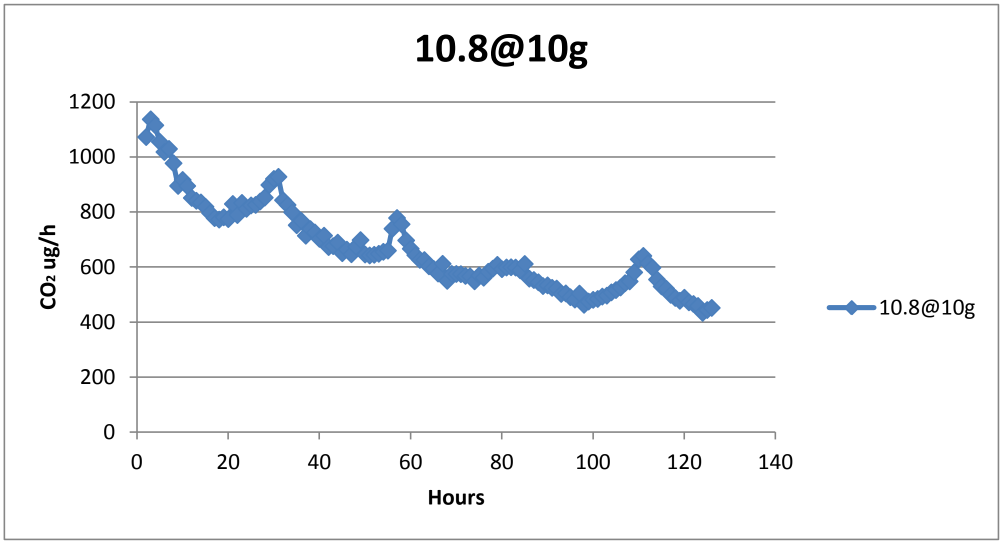


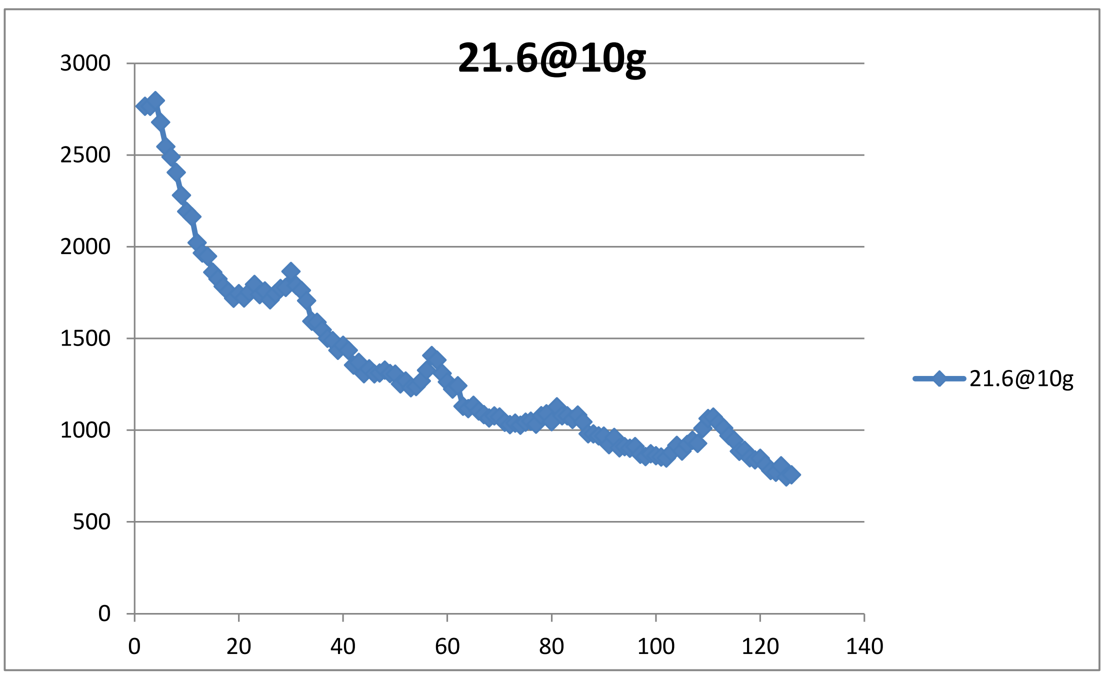

10.8@2.5g
500
450
400
350
300
250
200
150
100
50
0
CO₂ ug/h
0
20
40
60
80
100
120
140
Hours
10.8@2.5g

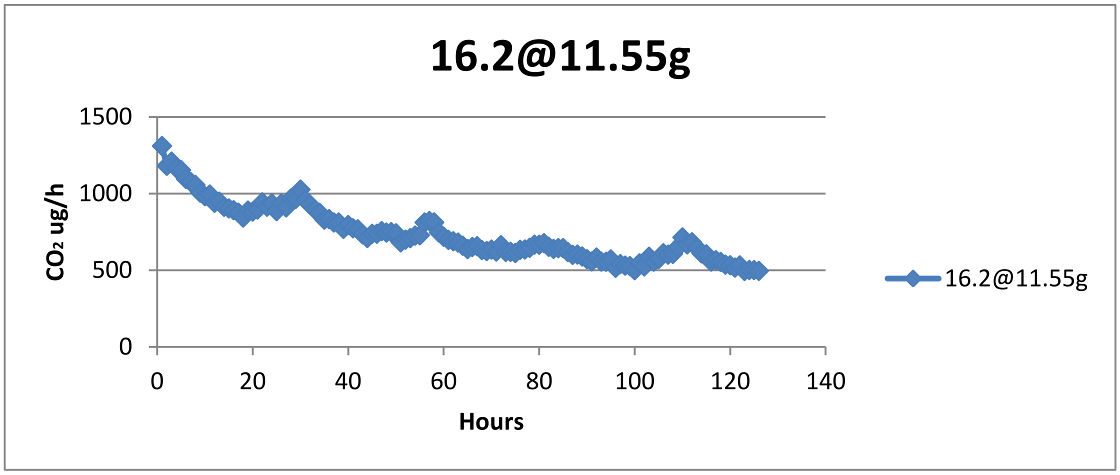

21.6@2.5g
800
700
600
500
400
300
200
100
0
CO₂ ug.h
0
50
100
150
Hours
21.6@2.5g


8.56@6.25g
1000
800
600
400
200
0
CO₂ ug/h
0
20
40
60
80
100
120
140
Hours
16.2@6.25g

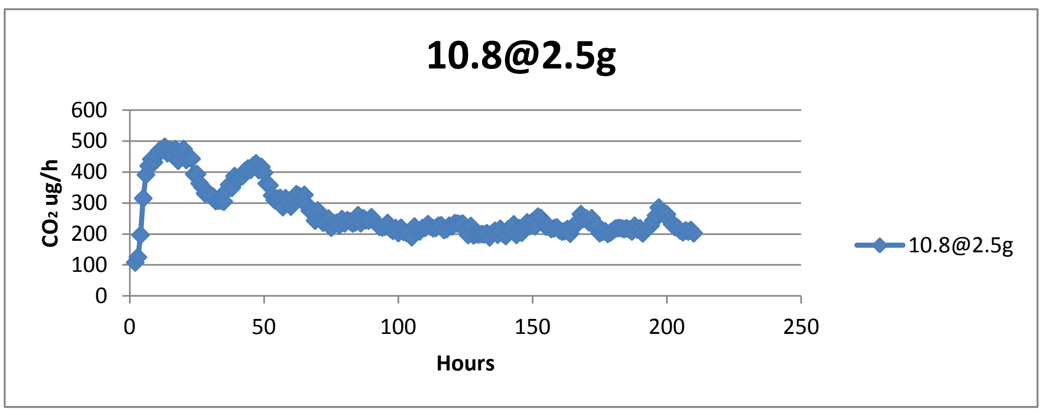

23.2@6.25g
2000
1500
1000
500
0
CO₂ ug/h
0
50
100
150
Hours
23.2@6.25g

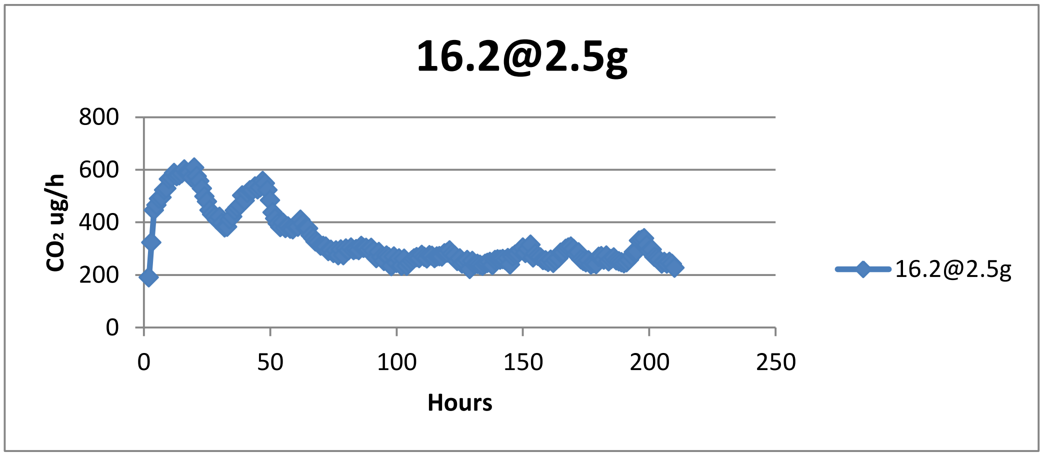

10.8@10g
1200
1000
800
600
400
200
0
CO₂ ug/h
0
20
40
60
80
100
120
140
Hours
10.8@10g


21.6@10g
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
21.6@10g

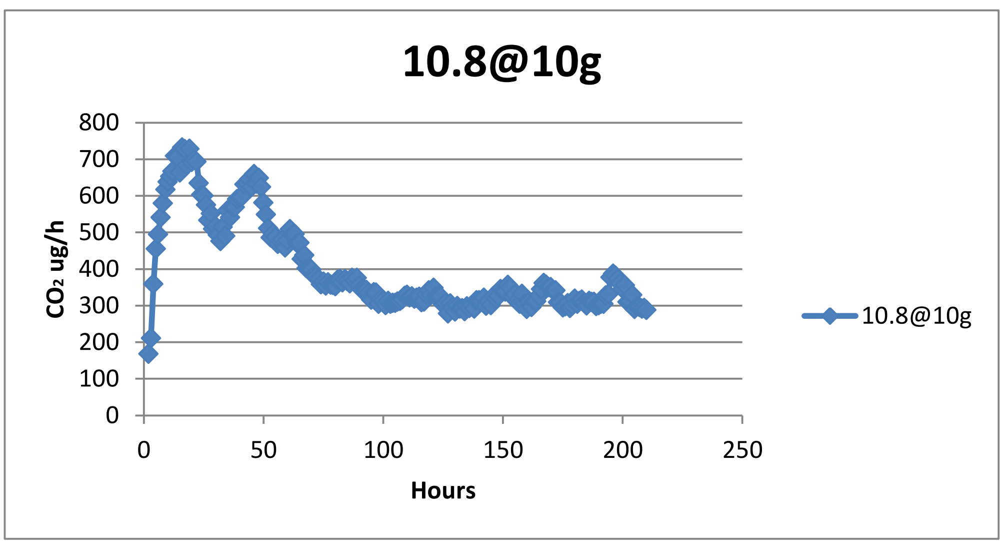


Forest soils after freeze-thaw

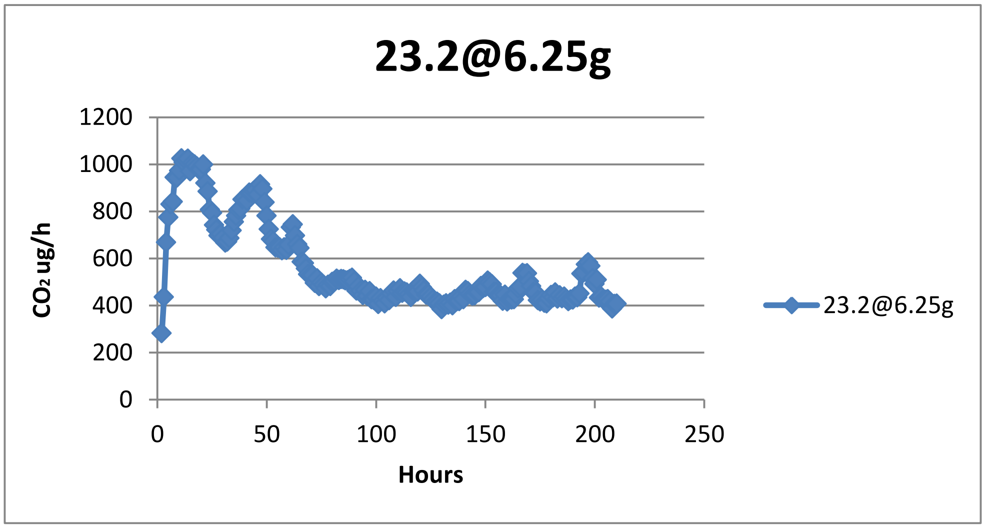




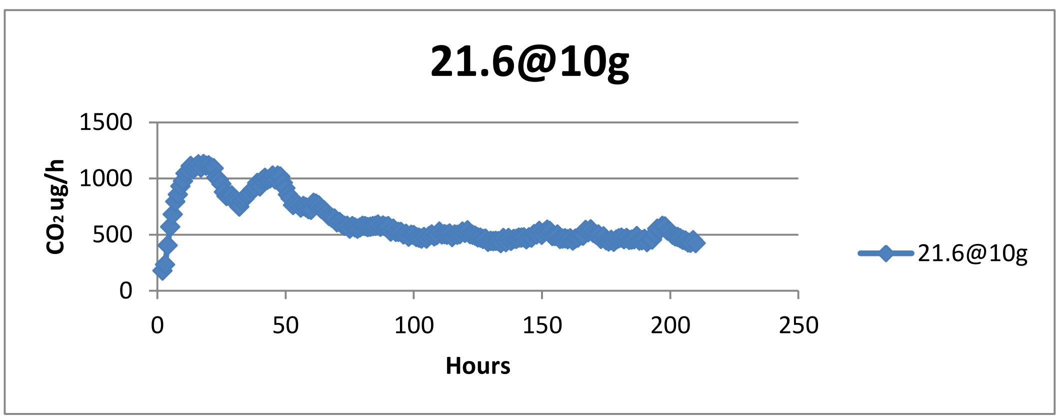
16.2@2.5g
800
600
400
200
0
CO2 ug/h
0
50
100
150
200
250
Hours
16.2@2.5g



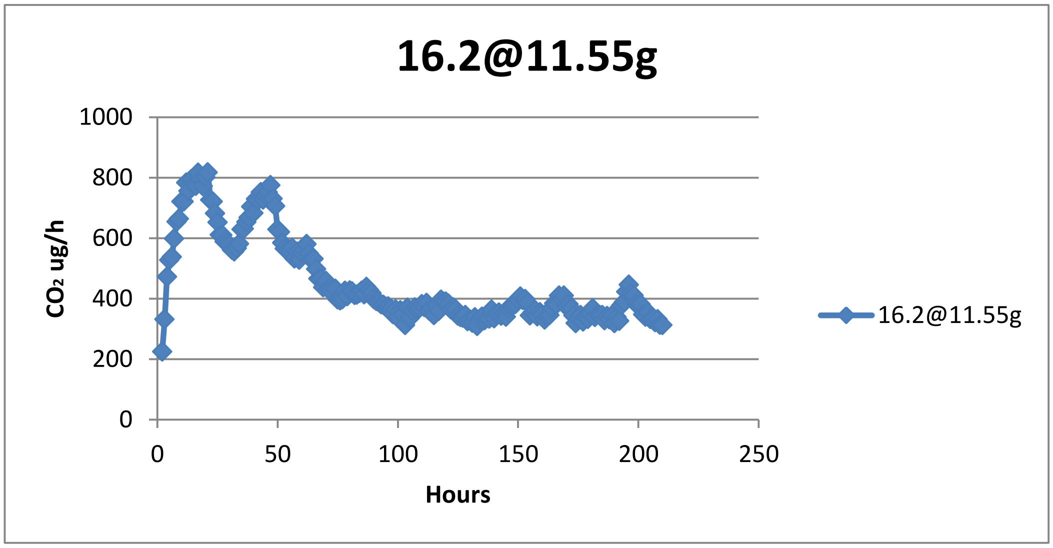
10.8@10g
800
700
600
500
400
300
200
100
0
CO2 ug/h
0
50
100
150
200
250
Hours
10.8@10g

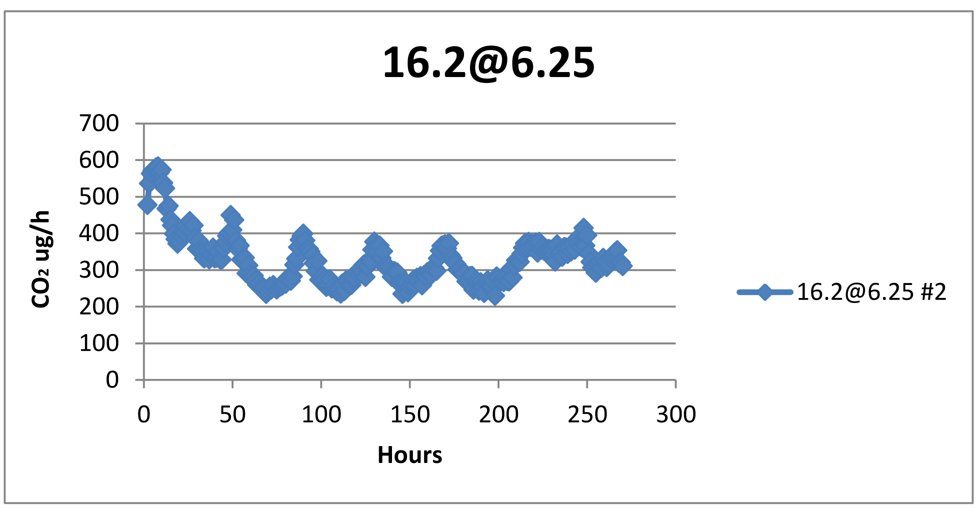


23.2@6.25g
CO2 ug/h
1200
1000
800
600
400
200
0
0
50
100
150
200
250
Hours
23.2@6.25g



21.6@10g
CO2 ug/h
1500
1000
500
0
0
50
100
150
200
250
Hours
21.6@10g


16.2@11.55g
1000
800
600
400
200
0
CO₂ ug/h
0
50
100
150
200
250
Hours
16.2@11.55g


16.2@6.25
700
600
500
400
300
200
100
0
CO₂ ug/h
0
50
100
150
200
250
300
Hours
16.2@6.25 #2